THE ROLES OF ALLELOPATHY, MORPHOLOGY AND GENETIC DIVERSITY IN THE INVASION OF SWALLOW-WORT SPECIES IN NEW YORK STATE

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ABSTRACT

Vincetoxicum rossicum (pale swallow-wort, PSW) and *V. nigrum* (black swallow-wort, BSW) are perennial herbaceous vines native to Europe that have become invasive throughout New York, neighboring regions of Canada, and the northeastern US. Introduced over a century ago, the two species have spread rapidly in recent decades. Established swallow-wort plants, with root-to-shoot biomass ratios of 6:1 and the ability to produce thousands of seedlings each season, are very difficult to control effectively using conventional chemical or cultural methods. Furthermore, swallow-worts are adept at modifying invaded habitats, favorably altering fungal mycorrhizal communities and displacing endemic flora and fauna. Compared with other serious invasive plant species, relatively little is known about the biological and ecological attributes that allow swallow-worts to be successful invaders. The research presented in this thesis seeks to address several important questions related to their invasiveness, namely their allelopathic potential, and the magnitude of genetic and morphological variation of populations found in several regions of New York differing in soil type and climate.

There have been reports in the literature indicating that these two swallow-worts are allelopathic and subsequently better able to out-compete and displace native flora. In this study, competition assays in agar media using seedlings demonstrated that SW root exudates - and particularly those of PSW - can cause significant root length reductions (e.g. 40% for butterfly milkweed) and germination rate decreases (e.g. 30% for lettuce) of indicator species. Bioassays using dried, field-collected tissue debris in a soil:sand growing media demonstrated that leachates from SW leaf tissues caused a negative effect on the growth of indicator plants: 40% and 50% reductions from BSW and PSW tissues, respectively. There was a significant dose

response, with 1 g of tissues resulting in significantly ($P < 0.05$) greater negative effects than 0.5 g of tissue. Furthermore, tissue leachates of the two species were self-inhibitory. BSW tissues decreased root growth (50%) of PSW indicators, but PSW tissues stimulated both root growth (38%) and germination (43%) of BSW indicator plants. A stimulation rather than inhibition of seedling growth was also observed for one of seven indicators (lettuce) in the agar root exudates experiment. The allelopathic capacity of the swallow-wort species was not significantly greater than that of a related non-invasive native species (common milkweed).

In addition to characterizing genetic diversity levels in introduced swallow-wort populations, we sought to identify whether genotype or environment (i.e. soil and climate) best explained the morphological variations observed in swallow-wort populations in New York. We found that the two species are monophyletic, but that intra-specific genetic similarity was high (mean = 0.79; Nei & Li's coefficient of genetic similarity). Genetic diversity did not explain morphological patterns in swallow-wort populations. Rather, adaptive morphological traits of invasive swallow-worts were correlated with soil pH (plant height: $\beta_1 = -0.67$, $r^2 = 0.5$, $P < 0.05$) and precipitation levels (plant height: $\beta_1 = 0.51$, $r^2 = 0.52$, $P < 0.0037$; leaf shape: $\beta_1 = 0.55$, $r^2 = 0.52$, $P < 0.05$). Also, in comparison with sites invaded by BSW, sites with PSW infestations were characterized by significantly ($P < 0.05$) higher soil pH (13%), Ca (475%), and Al (369%) levels.

The substantial inhibitory effects of swallow-wort tissue leachates are likely to have profound implications for management and restoration of invaded sites. However, our results suggest allelopathy is not a dominant factor explaining the invasiveness of the two species. Given that intra-specific genetic diversity of swallow-wort populations was lower than expected, the role of plant acclimation to environment in explaining swallow-wort invasion requires further study.

BIOGRAPHICAL SKETCH

Cameron Douglass was born January 30, 1980 in Kathmandu, Nepal to Bill and Ashton Douglass. He has two older sisters, Cassia and Sarah, and currently shares his life with his beloved wife Stephanie. His father was a diplomat in the US Foreign Service and so he spent his childhood living in Indonesia and Washington DC. In 1993 his family moved to Addis Ababa, Ethiopia, and it was at the International Community School there that he first developed his passion for science and plants. An adolescence spent in the midst of unfamiliar cultures and languages led to a deep respect for the boundless appeal of nature and its study.

In the fall of 1998 Cameron enrolled at the College of the Atlantic (COA) in Bar Harbor, Maine. COA awards only a single degree (BA Human Ecology) and its educational philosophy is predicated on the interdisciplinary study of human relationships to the natural world. This environment fueled Cameron's growing desire to pursue plant sciences, leading to an internship in the summer of 2001 with The Mountain Institute studying the cultivation of Giant stinging nettle by hill tribes in eastern Nepal. His undergraduate thesis compared the potential for allelochemical-mediated stimulo-deterrent diversionary strategies in Maine's potato crops and maize systems in eastern Kenya.

Cameron enrolled at Cornell University's graduate program in the fall of 2005 seeking his M.S. in horticulture with a focus on weed ecology under the guidance of Drs. Leslie Weston and David Wolfe.

This is dedicated to Stephanie - for inspiring me.

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Most significantly, I must acknowledge my committee members, Drs. David Wolfe and Toni DiTommaso for their guidance and patience. I am especially grateful to Dr. Wolfe, my major advisor, for his willingness to take me on and invest in me as a student. I would not be writing this thesis without the trust, support and encouragement of Dr. Leslie Weston, who gave me the chance to prove myself when few others did.

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The past two years would have been much duller and more difficult to endure without Chad's friendship, thank you for your humor and for listening. I of course owe much to my family - especially my parents for opening my eyes, challenging me, and always believing in me. Life is much more enjoyable and thrilling when you have such great champions on your side … *Maya namarnu hos.*

And to my wife Stephanie, you have shown me what is important in life. You have always supported me and pushed me, given me confidence. Knowing that you are by my side gives me the strength to push myself over the hurdles.

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Black and Pale Swallow-wort (*Vincetoxicum nigrum* and *V. rossicum*): Studies on the Biology and Ecology of Two Invasive, Exotic Vines in New York

Black and pale swallow-wort are invasive perennial plants that were introduced one hundred years ago into North America. Their invasion has been centralized in New York State, with neighboring regions of southern Canada and New England also affected. The two species have typically been more problematic in natural areas, but are increasingly impacting agronomic systems such as horticultural nurseries, perennial field crops, and pasture lands. While much of the literature reviewed herein is focused on the biology and management of the swallow-worts, conclusions are also presented from research assessing at the ecological interactions that occur within communities invaded by the swallow-wort species. In particular, we propose that the role of allelopathy and the relationship between genetic diversity, morphological variability, and environmental characteristics of infested sites could be significant in explaining the aggressive nature of swallow-wort invasion in New York. Findings from the literature suggest that the alteration of community-level interactions by invasive species, in this case the swallow-worts, could play a significant role in the invasion process.

INTRODUCTION

Black and pale swallow-wort (BSW; PSW) are invasive herbaceous perennial vines that were introduced over a century ago into North America. Currently both species are on statewide lists of banned or prohibited plant species in Connecticut, Massachusetts and New Hampshire, and BSW is classified as a 'noxious' weed in Vermont (USDA Plants Database 2008). Like many invasive species, the two

swallow-worts (SWs) exhibit numerous attributes of ideal weeds (Baker 1974). That is, they are strong competitors for available and sometimes scarce resources, are prolific reproducers, and can significantly alter invaded habitats (Ernst and Cappuccino 2005; Greipsson and DiTommaso 2006; Smith et al. 2006; Smith et al. 2008).

Unlike some of the more infamous plant invaders, SWs produce inconspicuous flowers and their often prostrate growth habit allows them to easily blend in with intact vegetation. SWs often persist largely unnoticed by landowners or managers until they are well-established and have displaced resident vegetation (Lawlor 2003; West and Fowler 2008). Mature vines can grow to several meters in length, and infestations are generally monocultural, existing as dense, impenetrable thickets of intertwined vines (hence the common name dog-strangling vine) (DiTommaso et al. 2005b; Sheeley and Raynal 1996). Most significantly, cultural control methods to effectively reduce mature infestations are not available at present. Current recommendations for control are limited to the use of broad-spectrum herbicides and mechanical controls that must be repeated both during the growing season and annually for several years. Unfortunately, even these laborious and expensive strategies only provide reliable control of smaller satellite populations (Averill et al. 2008; Lawlor and Raynal 2002; Weston et al. 2005).

This paper provides a review and synthesis of the biology and ecology of the two SW species as related to their invasiveness, including (i) a discussion of characteristics and patterns of their respective invasions and infestations in New York, (ii) possible reasons for their invasion success, (iii) potential management strategies, and (iv) future research priorities.

TAXONOMY

*Pale swallow-wort (*Vincetoxicum rossicum *(Kleopow) Babar.* = Cynanchum rossicum *(Kleopow) Borhidi) and black swallow-wort (*Vincetoxicum nigrum *(L.) Moench* = Cynanchum nigrum *(L.) Pers.* = Cynanchum louiseae *(L.) Kartesz & Gandhi)* are generally placed in the Asclepiadaceae (Gleason and Cronquist 1991; USDA Plants Database 2008). Recent work however suggests that the species should more accurately be placed in the Apocynaceae (SJ Darbyshire, Personal communication). In Europe there is evidence of successful hybridization between *V. rossicum* and another relative, white SW (*V. hirundinaria* Medik.) (Lauvanger and Borgen 1998). This evidence not only contributes to taxonomic confusion regarding identification of SWs, but also points to the potential for pale and BSW to hybridize (DiTommaso et al. 2005b).

There remains a great deal of confusion as to the correct taxonomic placement of the invasive SW species, with some taxonomists placing them under the genus *Cynanchum* and others in the genus *Vincetoxicum*. Given that there are a number of native North American plants in the genus *Cynanchum* (22 according to the USDA Plant Database), DiTommaso et al. (2005b) proposed that *Vincetoxicum* should be used solely for the alien species in order to denote their old world origins. This view is shared by Liede (1996), who, based primarily on the presence of unique alkaloids and glycosides, separates *Vincetoxicum* from *Cynanchum* and places pale and BSW within the genus *Vincetoxicum* (also Liede and Tauber 2002).

From a review of the literature, no studies have been performed to determine the ploidy level or chromosome numbers for SW populations in the United States, and other evaluations that have been performed were limited in their geographic focus. A chromosome count of $2n = 22$ was reported for PSW plants in Ottawa, Ontario, Canada (Moore 1959). Chromosome counts for BSW plants vary from $n = 11$ in

Spain (Diosdado et al. 1993) to $2n = 22$ and $2n = 44$ for two populations in Italy (Aparicio and Silvestre 1985; Moore 1959). At this time, we have no information on chromosome numbers of SW populations in the United States, valuable information to more fully describe the genotypic relationships among and between these two species.

PSW is native to eastern regions of the Ukraine and southwestern portions of Russia north of the Black Sea and Caucasus. BSW is endemic to southwestern Europe, particularly regions of the Iberian Peninsula, southern France and northern Italy (DiTommaso et al. 2005b). In their native ranges, the two species are relatively rare, with scattered patches of 3-15 stems of BSW found to be typical of a native populations in southern France (F Lawlor *in* DiTommaso et al. 2005b; LR Milbrath, Personal communication; Tewksbury et al. 2002). While PSW has been reported as invasive in one case in Norway, the third *Vincetoxicum* species, *V. hirundinaria*, is actually much more widespread and has greater invasive potential in Europe than the two species that are problematic in North America (Lauvanger and Borgen 1998).

REPRODUCTIVE BIOLOGY AND PHENOLOGY

Flowering can begin as early as mid to late May for PSW populations in Central New York and will peak several weeks later in early to mid June (Sheeley 1992). Floral development can be delayed by ten days in populations farther north in New York, and by up to four weeks for populations in Ontario and other regions of southern Canada (DiTommaso et al. 2005b; Lawlor 2000). BSW flowering tends to peak in mid to late June; though in shadier sites it can be delayed by up to a month (DiTommaso et al. 2005b). Lumer and Yost (1995) found that flowers typically remain open 6-8 days, a period longer than many related species, which the researchers attributed to a complicated pollination process. The SW species are self-compatible, but are also insect-pollinated by a variety of fly, ant, bee, wasp and beetle

species (DiTommaso et al. 2005b). For BSW, insect pollination occurs primarily via non-specialized flies with shorter tongues (e.g. *Pollenia rudis*, *Phaenecia sericata* or *Sarcophaga* spp.), with the dark color of BSW flowers and their slightly fetid odor attracting pollinators (Lumer and Yost 1995).

Fruit development for PSW typically begins in early June in Central New York, with maturity occurring four to five weeks after flowering, and finally dehiscence peaking at the end of July. Development in BSW is normally two to four weeks slower (DiTommaso et al. 2005b; Lawlor 2000). There appears to be a physiological dormancy requirement for PSW seeds specifically, and this likely applies as well to BSW. Though minimal germination is often found experimentally with PSW without subjecting seeds to a cold stratification period, greater germination occurs in seeds subjected to a period of stratification (Cappuccino et al. 2002; DiTommaso et al. 2005a).

While some studies have found a positive correlation between seed size and the probability of germination in PSW (DiTommaso et al. 2005a), others have reported no correlation between seed size and germinability (Cappuccino et al. 2002; C Douglass, Unpublished data). Cappuccino et al. (2002), however, did find that seed size in PSW was positively correlated with final dispersal distance. This effect was weaker in a later study (Ladd and Cappuccino 2005) though the positive trend did generally hold true. While these authors also found that larger seeds tended to produce taller seedlings during the first growing season, they concluded that there was a nominal advantage in survivorship associated with greater initial seed weight during three growing seasons.

SW species produce polyembryonic seed, which is estimated to occur in 45-75% of pale swallow-wort seeds (Sheeley 1992; St. Denis and Cappuccino 2004). Our own work indicates that the occurrence of polyembrony is much lower in BSW in

comparison to PSW, with the probability of a PSW seed being polyembryonic roughly ten times greater than for a BSW seed (C Douglass, Unpublished data). Research suggests that polyembryonic seeds are more successful than monoembryonic seeds in undisturbed habitats and in the absence of strong competitors - which often occurs in disturbed areas (Cappuccino et al. 2002; Ladd and Cappuccino 2005). However, in a recent 3-year field study in central New York, Hotchkiss et al. (2008) reported that polyembryonically-derived PSW plants were not afforded a survival or growth advantage over plants derived from single embryo seeds under both high and low light environments within a forest site.

PSW has a stout and often large root crown that produces perennating buds and extensive, fleshly roots (DiTommaso et al. 2005b). Many plants also possess a horizontal, woody rhizome, though this structure does not appear to substantially facilitate dispersion of the plants (Cappuccino 2004; Weston et al. 2005). The root-to-shoot biomass ratio of PSW can be substantial, up to 6.7 in New York soils that contained beneficial arbuscular mycorrhizal fungal (AMF) species (Smith 2006). Root structures in BSW are similar but tend to be thicker and more fibrous, and rhizomes in this species are reported to contribute more significantly to population expansion (DiTommaso et al. 2005b; Lumer and Yost 1995). For example, Lumer and Yost (1995) often found adjoining plants that were connected by "horizontal underground stems" growing at a depth of nearly 50 cm.

Both SW species, and PSW in particular, have high seed output potentials. At a heavily infested site in northern New York, Smith (2006) reported a potential seedling output of 63,439 seedlings m^{-2} when polyembryonic offspring were taken into account. However, it is not clear whether newly emerged seedlings or older seedlings contribute more to the expansion of SW patches. Averill et al. (2006) reported that for both SW species the majority (79%) of seedlings found in the spring were at least one

year-old, while only 20% were produced from newly germinated seeds. On the other hand, Ladd and Cappuccino (2005) found that when they planted (buried 1 cm) over-wintered PSW seeds in an experimental old field, 71% of the seeds germinated in the first year. SW seeds generally mature dormant, and while experimentally germination levels can be doubled in stratified seeds, the nature and extent of this dormancy is unknown (DiTommaso et al. 2005a, 2005b; Lumer and Yost 1995).

INTRODUCTION AND CURRENT DISTRIBUTIONS

The earliest North American collection of PSW was made in 1885 from Victoria, British Columbia (Sheeley 1992). The earliest collection in the United States was six years later (1891), simultaneously recorded in both Monroe and Nassau Counties in New York. The first specimen of BSW in North America was collected in Ipswich, Massachusetts in 1854 (DiTommaso et al. 2005b; Sheeley and Raynal 1996).

The most likely source of introduction of both species was importation as specimens for botanical or estate gardens (DiTommaso et al. 2005b; Lauvanger and Borgen 1998; Sheeley 1992). In Gray's 1867 *Manual of Botany* BSW was cited as a garden escape in Cambridge, MA (Anonymous 1871). By 1871 there was a report of the plant "running wild" along a road in the "Flatlands", in modern day Brooklyn, New York. Eleven years later, it was described as naturalized in West Point and New Rochelle, NY (Bailey 1882; Day 1881). For many years the two SW species were cultivated and sold as ornamental plants, though this is no longer common (DiTommaso et al. 2005b; Monanchino 1957).

Current Distribution. PSW invasion in North America is centralized in upstate New York, specifically the Finger Lakes Region and the Lake Ontario region in both the United States and southern Canada (DiTommaso et al. 2005b). There are additional

extensive populations throughout Long Island, NY and other states in the Northeast, and there have been isolated reports of plant sightings throughout the Mid Western states (Weston et al. 2005). BSW has a wider distribution longitudinally, with populations reported as far west as Kansas, Nebraska, Minnesota and even California. However, its invasion is also centered in New York, with the most severe infestations found in the Hudson River Valley, and into Massachusetts and Connecticut (DiTommaso et al. 2005b). The wider distribution of BSW has been attributed to its apparent ability to adapt to more severe climatic conditions than encountered in its native Mediterranean range, unlike PSW which has largely remained within its predicted climatic boundaries in its introduced range (DiTommaso et al. 2005b).

Both SW species are typically found in habitats with temperature ranges in the winter of 0.7 °C to -11 °C and in the summer of 20.7 °C to 26.4 °C, while mean annual precipitation levels in these areas range from 776 mm to 1206 mm (DiTommaso et al. 2005b). In contrast, annual temperatures in Ukraine (PSW's native range) vary from –8 °C to 24 °C with a mean precipitation of 629 mm (World Meteorological Organization 2008). Temperatures in southwestern France (part of BSW's native range) vary from 2 to 28 °C with 668 mm of precipitation, and in northeastern Spain, the climate varies from 2 to 31 °C with only 317 mm of precipitation.

IMPACTS

PSW has invaded sensitive and rare alvar communities both in eastern Ontario, Canada and in Jefferson County, NY, and has displaced sensitive endemic flora and fauna there (DiTommaso et al. 2005b). A survey in the affected areas revealed a significant negative correlation between PSW cover and the number and diversity of previously common grassland bird species (DiTommaso et al. 2005b). Ernst and

Cappuccino (2005) found fewer arthropods both dwelling on PSW plants and ground-dwelling insects adjacent to sampled plants. The authors concluded that the decline in old-field arthropod populations because of the invasion of SW could negatively impact bird and small mammals that also depend on insects for food.

Lawlor (2000) reported that habitats of the Hart's tongue fern (*Asplenium scolopendrium* L. var. *americanum* (Fern.) Kartesz & Gandhi), a rare plant species native to regions of New York, have been invaded by PSW. Similarly, PSW has invaded sites at The Nature Conservancy's Mashomack Preserve on Shelter Island, NY where the federally listed endangered species sandplain gerardia (*Agalinis acuta* Pennell) is found (M Scheibel, Personal communication). BSW is a less intensively studied species and thus few targeted studies have been carried out to assess the ecological impacts of its invasion. One study found that *V. nigrum* threatens the survival of the endemic Jessop's milkvetch (*Astragalus robbinsii* (Oakes) A. Gray) along the banks of the Connecticut River in Windsor, Vermont, one of only three locations in which the plant is known to remain (DiTommaso et al. 2005b).

While the SWs have had a substantial negative impact in a variety of natural areas, the species pose a substantial and looming threat to New York's important agricultural industry. The detection of PSW plants in no-till corn and soybean fields is problematic given the relative difficulty of controlling either of the SW species effectively with commonly used herbicides in crop systems (DiTommaso et al. 2005b; Lawlor 2003; Weston et al. 2005). There have been numerous reports of landowners abandoning horse pastures due to unmanageable infestations of PSW, possibly due to the physical obstruction posed by dense SW stands or the suspected toxicity to animals of plant tissues (Lawlor 2003; Weston et al. 2005). A feeding trial with fresh PSW plant material resulted in the death of a goat from suspected cardiac arrest four days

after the last tissue treatment, which seems to support evidence from Scandinavia of sheep avoiding grazing on PSW plants (DiTommaso et al. 2005b; Haeggstrom 1990).

The New York State Forest Owner's Association and many foresters have claimed that SW infestations in understories are also compromising forest regeneration (Lawlor 2003). Horticultural nursery owners and Christmas tree producers have also experienced SW infestations and reported that due to a lack of effective control methods land abandonment was often the only solution (Weston et al. 2005). Indeed, several orchard owners east of Rochester, NY cited PSW as one of their most problematic weed species (A Fowler, Personal communication; Lawlor 2003).

Swallow-worts and Monarch butterflies. The potential for both SW species to serve as fatal hosts for Monarch butterflies (*Danaus plexippus* L.), a condition in which adults lay eggs on the plants but the larvae do not survive, has been well reported (Casagrande and Dacey 2001; DiTommaso and Losey 2003). More recently, Casagrande and Dacey (2007) found that in fields with little or no common milkweed (*Asclepias syriaca* L.), the density of eggs found on BSW stems was five times greater than that found in a more diverse old-field site with abundant common milkweed. Although there have been studies that have questioned whether SWs play a significant role as fatal hosts for Monarch butterflies (Mattila and Otis 2003), it is likely that through the competitive displacement of common milkweed populations, the two SW species could ultimately pose a serious threat to Monarch butterfly populations in infested areas (DiTommaso et al. 2005b; Tewksbury et al. 2002).

MANAGEMENT

Cultural Control. Manual methods can be effective at controlling established patches of perennial weeds (Radosevich et al. 1997; Ross and Lembi 1999). However, given that both SW species can rapidly re-grow from buds on the root crown, mowing, tillage, and other control strategies are less effective against established populations (Lawlor and Raynal 2002; Weston et al. 2005). Mowing can be effective at containing invasions when timed to suppress seed production, but must be repeated for the duration of the growing season as plants tend to re-grow more rapidly than non-mowed plants and produce seed at atypically young stages of growth (C Douglass, Personal observations). With repeated mowing, root reserves are depleted and eventually the affected plants are less competitive (Radosevich et al. 1997). Because of their tall, brittle stems, SWs are particularly sensitive to trampling, which has resulted in a substantial reduction of PSW patches in some localized fields (DiTommaso et al. 2005b).

Chemical Control. There are several herbicides that provide relatively effective control of the two SW species when applied postemergence (Averill et al. 2008; Lawlor 2002; Weston et al. 2005). Foliar applications are generally more difficult to apply than cut stem applications because of the intertwining growth habit of the SWs and high patch densities at maturity, but are generally more effective (Lawlor and Raynal 2002). Lawlor and Raynal (2002) found that foliar applications were significantly more effective at controlling plants in shaded plots than drier, full sun plots.

In particular, the most effective chemical treatments were glyphosate (10.4 kg ai ha^{-1}) applied at an early stage of flowering and triclopyr (2.6 kg ai ha^{-1}) applied at early fruit formation, both of which resulted in a 73% reduction in cover (Lawlor and

Raynal 2002). Recent work has demonstrated that glyphosate applied at a much lower rate (1.79 kg ai ha^{-1}) was just as effective (77% reduction in cover when applied in late June) as higher rates, and more effective overall than triclopyr alone or combinations of triclopyr and 2,4-D or dicamba and 2,4-D (F Lawlor, unpublished data in Weston et al. 2005). In any case, an adequate surfactant must be included in postemergent foliar applications so that uptake is maximized, particularly because of the waxy cuticle present on the leaf surfaces and stems of both species (Radosevich et al. 1997).

Biological Control. To date, research on the biological control of the two SW species with insects has been limited (Tewksbury et al. 2002; Weston et al. 2005). Since most larval stages of insects do not thrive on foliage of the alkaloid-containing leaves of *Vincetoxicum*, effective biocontrol with insects presents a strong challenge to researchers in finding an herbivorous insect for specific long-term control (Christensen 1998; Tewksbury et al. 2006). Potential pathogens of these two species have not been found, although several pathogenic organisms do infect members of the milkweed family (Weston et al. 2005). Recently, the USDA's Agricultural Research Service (ARS) Laboratory located in Ithaca, NY and headed by L.R. Milbrath initiated a biocontrol program targeting both of these invasive species. The search for potential biocontrol agents has focused primarily in Europe and Eurasia. The criteria for biocontrol programs specify that the candidate organism is able to be propagated in culture, successfully released and established in New York, and remain specific to the SWs, rather than exhibiting generalist behaviors (Milbrath and Gibson 2006). Given the lack of success from chemical and cultural tactics to date, biocontrol of these two SW species might offer the greatest potential for successful long-term control in North America (Tewksbury et al. 2002).

CHARACTERISTICS AND PATTERNS OF INVASION

Previously, Cappuccino et al. (2002) demonstrated that 50% of PSW seeds landed within 2.5 m of their release points. However, seed weight was inversely correlated with dispersal distance so that lighter seeds dispersed further. More recently, Ladd and Cappuccino (2005) found that 83% of seeds produced by PSW plants landed directly beneath the parent plant. Moreover, 51% of seeds placed on the soil surface germinated and resulted in emergent seedlings while 71% of seeds buried at a soil depth of 1 cm resulted in emergent seedlings. This high rate of emergence most likely facilitates the ability of these two SW species to rapidly and successfully establish satellite populations (Ladd and Cappuccino 2005). First-year PSW seedlings also have unusually high survivorship (71-100%) when compared with many other herbaceous plant species. This latter study is remarkable in that it was performed in an undisturbed old field community, suggesting that while the SWs are often invasive in disturbed habitats, they can also invade well-established and intact natural plant communities (Ladd and Cappuccino 2005).

Direct competition with monocots was found to significantly decrease the average size of PSW seedlings, and especially for seedlings produced from larger-sized seeds (Cappuccino et al. 2002). All but two of the seedlings grown in the presence of grasses were smaller than expected, while 90% of those grown in the absence of competition were of above average size. In particular, the production of polyembryonic seedlings did not lead to a significant competitive advantage in the presence of neighbors but was beneficial in the absence of competition. Polyembryony may be most beneficial in disturbed habitats or areas where native vegetation has been removed because of the enhanced ability of multiple seedlings to successfully establish (Cappuccino 2004; DiTommaso et al. 2005a).

PSW can have strong drought tolerance as shown by the relatively low water tension levels reported for the species (Ψ = -0.062 mPa at midday and -0.079 at pre-dawn) (DiTommaso et al. 2005b). This may be due, in part, to its extensive root system and waxy leaf cuticle, suggesting that this species can effectively tolerate environmental stresses that may reduce the vigor and performance of associated plant species (DiTommaso et al. 2005b). Recent research on PSW has demonstrated that soils at sites invaded by this species have greater AMF inoculum potentials than adjacent, uninvaded sites, and with host plants showing significantly greater growth when grown in the presence of locally-associated microbial communities than non-local communities (Greipsson and DiTommaso 2006; Smith 2006; Smith et al. 2008). These authors proposed that this alteration of the soil microbial community could facilitate the ability of the SW species to displace resident flora. Lumer and Yost (1995) hypothesized that the ability of BSWs to self-pollinate, along with their reported capacity to spread vegetatively and the lack of a substantial dormancy requirement, would make this species a successful colonizer and an invasive threat.

*Habitat and Environmental Variability***.** Sheeley (1992) and Lawlor (2000) both found a positive correlation between irradiation received at the canopy level and PSW stem density, with densities five times greater in full sun than in deep forest shade. However, Sheeley (1992) also reported that internode and stem lengths were greater in shaded sites than sunny sites, but that since the stem weights did not differ this indicated that there were differences in stem diameter between the sites. Smith (2006) did not find a consistent correlation between stem densities and light availability in any habitat type, although plants in higher light plots produced more flowers per plant and had an overall higher reproductive biomass. Moreover, seeds produced by plants in the lower light plots were significantly more likely to posses dormant or non-viable

embryos (Smith 2006; Smith et al. 2006). Smith et al. (2006) reported a seasonal variation in seedling stem densities of PSW in a northern New York State site, with an almost five-fold decrease between late July and August.

In Central New York, PSW plants are normally found on shallower soils over limestone bedrock or deep, well-drained silt-loam soils in wooded ravines, calcareous cliffs, talus slopes, alluvial woods, pastures and grasslands (DiTommaso et al. 2005b; Weston et al. 2005). The plants exhibit a wide tolerance to light and moisture conditions, but appear to be particularly successful and aggressive on shallow, droughty soils or deeper silt loams with partial to full sun (Lawlor 2002; Lawlor and Raynal 2002; DiTommaso et al. 2005a; Smith 2006). DiTommaso et al. (2005a) found that growth and fecundity of both SW species was substantially greater in open, sunny sites or gaps in the forest understory. Similarly, Sheeley (1992) found that PSW plants in heavily shaded sites produced both fewer flowers and fewer seeds. Recent work by Hotchkiss et al. (2008) in a central New York forest supports previous findings of significantly greater growth of PSW plants in forest gaps relative to shaded forest understories. In preliminary findings from several field sites in New York, Averill et al. (2006) found that the number of tillers per plant for several populations of PSW increased 45% at open field sites but only 19% in forest understory sites during two growing seasons. BSW appears to share comparable habitat preferences, but is often limited to sunny, open field sites rather than shaded forest sites (Lumer and Yost 1995).

Invasiveness. Cappuccino (2004) found that smaller-sized patches (both 1 and 9 plants) of PSW had higher reproductive success (measured as maturation of follicles) in the second year of her study, and greater root-to-shoot biomass than larger-sized patches (81 plants). However, the larger patch produced three times more follicles and

thus had a greater net production of seed. When considered in the context of her earlier work (Cappuccino et al. 2002), the latest results suggest that in general the larger patches will produce large quantities of seed and that at least a portion of these seeds will lead to the successful establishment of pioneering satellite populations.

These smaller satellite patches appear to invest proportionally greater resources into root biomass, presumably to ensure establishment, before allocating resources to vegetative or reproductive structures (Cappuccino et al. 2002; Cappuccino 2004; Smith 2006). Indeed, Smith (2006) found that PSW had a significantly greater root-to-shoot biomass ratio than its close relative common milkweed. Moreover, when in competition with common milkweed, young PSW plants had greater overall reproductive output. While diminishing its competitive ability relative to common milkweed, this allocation of resources to reproduction could ensure the presence of a large seed bank from which satellite populations could be produced(Cappuccino 2004; Lockwood et al. 2007; Myers and Bazely 2003). Given that seed output of a single PSW population in central New York was 35,244 to 62,439 seeds/m^2 (the higher figure takes into account the proportion of polyembryonic seeds), the seed bank of this species can be quite large, and its impact over the course of several years significant (Smith 2006).

Once established, both SW species grow profusely and aggressively. Both SW species can rapidly alter the abiotic and biotic features of their understory and surrounding areas: decreasing sunlight penetration, increasing nutrient acquisition through large root biomasses, altering rhizosphere dynamics both through shifts in the arbuscular mycorrhizal fungal (AMF) community and the exudation of allelopathic chemicals (Greipsson and DiTommaso 2006; Lawlor 2002; Sheeley and Raynal 1996; Weston et al. 2005). Despite the increasing number of studies on the two invasive SW species, there have been no published investigations to date of three potentially

significant factors that may influence the invasiveness of SWs, namely: allelopathy by tissues and root exudates; adaptive morphological plasticity; and genetic diversity of introduced populations.

Allelopathy. Cappuccino (2004) first reported that SW root extracts inhibited the germination of radish seeds and showed broad anti-fungal activity. Furthermore, DiTommaso et al. (2005b) suggested that this purported allelopathic activity by root exudates/extracts could indirectly affect competitive interactions through the alteration of the structure of the rhizosphere community. An interaction between allelopathic activity and mycorrhizal associations may cause profound ecological effects on population dynamics of native species and diversity of affected plant communities (Roberts and Andersen 2001; Stinson et al. 2006).

A subset of exotic plant species competitively exclude and eliminate their neighbors in invaded "recipient" communities, but generally are found to coexist with neighbors in species-diverse systems in their native habitat. Allelopathy has been suggested as one of the mechanisms responsible for this success (Callaway et al. 2005, Inderjit et al. 2006a). Although allelopathy has not yet been clearly implicated in association with the field dominance of any invasive species, evidence suggests that many invasive species produce an array of secondary plant products or allelochemicals that are released through decomposition or directly exuded from roots or shoots into the soil rhizosphere (Inderjit et al. 2006b).

It is thought that both SW species might exhibit some allelopathic activity due to the high concentrations of secondary products found in *Vincetoxicum* spp. roots, stems and leaves (Capo and Saa 1989; Lee et al. 2003; Nowak and Kiesel 2000; Staerk et al. 2000 & 2002). In unpublished laboratory work, N. Cappuccino had demonstrated that foliage of PSW produces phytotoxins when these tissues are ground and their

chemical constituents extracted using water (N Cappuccino, Personal communication). Other investigators have postulated that the dense monocultural stands created by SWs following establishment may have resulted from the exudation of root-released allelochemicals that limit growth of neighboring species. The decomposition of foliage and stems underneath a SW stand may also contribute to seedling suppression from the effects of both allelopathy and limited light reaching the soil surface (Weston et al. 2005).

Empirical work quantifying the potential for allelopathic activity from *Vincetoxicum* root exudates and tissue leachates is needed to better understand the role that allelopathy might play in the competitive dominance of these two SW species. Once allelopathy has been confirmed, research priorities should focus on two areas: isolation of biologically active compounds; and biogeographically-based field work assessing the ecological role played by allelopathy in invasion by SWs.

Phenotypic and Genetic Diversity. Ellstrand and Schierenbeck (2000) proposed that hybridization (both inter- and intra-specific) could play an important role in enhancing the invasiveness of introduced species. In particular, they suggested that hybridization between populations of the same taxa could lead to adaptive evolution in cases where the species was intentionally introduced multiple times (resulting in a diverse gene pool), and that this process would occur only after a lag period. Given the evidence of hybridization occurring between *Vincetoxiucm* species, we suggest that the potential for evolutionary changes in invasive SWs is high considering their increasingly overlapping ranges (DiTommaso et al. 2005b; Lauvanger and Borgen 1998).

There is also evidence that the rapid evolution of plasticity for ecologically advantageous phenotypic traits is relatively common among invasive species, and that this may partially explain the success of many invasive species only after an initial lag

time during which necessary evolutionary adjustments have occurred (Pigliucci 2005; Richards et al. 2006). The potential roles of trait plasticity in the invasiveness of the two SW species are of great interest given reports of wide variation in reproductive and phenological traits among invasive SW populations (DiTommaso et al. 2005b; Lawlor 2000; Sheeley 1992; St. Denis and Cappuccino 2004).

Finally, Nevo (1988) and Nevo et al. (1984) found that plant species tend to be more genetically polymorphic if they occur in broader climatic, ecological or biotic spectra, both at the macro- and micro-geographic scale. Similarly, Agrawal (2001) predicted that species whose phenotypic traits are exposed to (and thus respond to) larger ranges of environmental stimuli will be more likely to influence both the ecology and perhaps evolution of that species' interactions in novel habitats. The characterization of genetic diversity and patterns of morphological variation among and between introduced SW populations should be a research priority. The success of future biological control programs and management of the species is dependent on knowledge of the genetic structure and the capacity of the species to be phenotypically plastic.

CONCLUSIONS

It has been proposed that it is possible to predict the invasiveness of particular plant species from life history traits, e.g. vegetative reproduction or seed size (Myers and Bazely 2003; Rejmanek and Richardson 1996). The two SW species fit many of these characteristics well, with one exception - at this time, we do not clearly understand the role of vegetative reproduction in the rapid spread of the two species (DiTommaso et al. 2005; Weston et al. 2005). Furthermore, the apparent ability of the species to become invasive in many locations in New York might indicate that swallow-worts have an enhanced propensity towards establishing in and becoming

invasive in novel habitats that are outside of their current range (Lockwood et al. 2007).

Myers and Bazely (2003) propose two key areas of study that are important in determining the invasiveness of a given species: (1) interactions between invasive species specifically (as well as the impact this has on existing community structure and function), and (2) the interactions between and evolution of plants and soil organisms. Given the high reproductive outputs of SWs, the unknown role of vegetative expansion, and the clear impacts that SW invasions have on both belowground and aboveground communities, it is vital to investigate the role that interactions between the SWs and the biotic communities in invaded habitats may play in the aggressive invasion characteristics of these two species (Averill et al. 2006; Griepsson and DiTommaso 2006; Mitchell et al. 2006; Smith 2006).

Since Elton's *Ecology of Invasion by Animals and Plants* (1958), the idea of "ecological resistance," that invasive species tend not to establish in intact native communities due to an inherent ecological balance, has been a frequently referenced foundation of invasion ecology. SW species often become well established and monocultural in habitats that appear to have previously been largely intact natural communities, including grasslands and forest understories (Ladd and Cappuccino 2005). It is especially because of their ability to succeed in colonizing intact ecological communities, that we strongly suspect that the two SW species could quickly become widespread and noxious. Educational outreach programs with landowners and natural area managers is critical to prevent further spread, as are ongoing studies related to their control and management.

REFERENCES

Agrawal A (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294: 321-326

Anonymous (1871) Bulletin of the Torrey Botanical Club 2: 43

Aparicio A, S Silvestre (1985) Numeros cromosomicos para la flora Espanola 422-434. Lagascalia 13: 318-323

Averill KM, DiTommaso A, Mohler CL, Morris SH, Milbrath LR (2006) Vegetative expansion and seedling fate of swallow-worts (*Vincetoxicum* spp.), introduced invasive vines in the northeastern US. Ecological Society of America Meeting Abstracts 91:16

Averill KM, A DiTommaso, SH Morris (2008) Response of pale swallow-wort (*Vincetoxicum rossicum*I to triclopyr application and clipping. Invasive Plant Science and Management (*In press*)

Bailey WW (1882) Bulletin of the Torrey Botanical Club 9: 106

Baker HG (1974) The evolution of weeds. Annual Review of Ecology and Systematics 5: 1-24

Callaway RM, Ridenour WM, Laboski T, Weir T, Vivanco JM (2005) Natural selection for resistance to the allelopathic effects of invasive plants. Journal of Ecology 93: 576-583

Capo, M, JM Saa. 1989. (-)-antofine: a phenanthroindolizidine from *Vincetoxicum nigrum*. Journal of Natural Products 52(2): 389-390

Cappuccino N (2004) Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos 106: 3-8

Cappuccino N, Mackay R, Eisner C (2002) Spread of the invasive alien vine *Vincetoxicum rossicum*: tradeoffs between seed dispersability and seed quality. American Midland Naturalist 148: 263-270

Casagrande RA, Dacey J (2001) Monarch butterfly (*Danaus plexippus*) oviposition on black swallow-wort (*Vincetoxicum nigrum*). Rhode Island Natural History Survey 8: 2-3

Casagrande RA, Dacey J (2007) Monarch butterfly oviposition on swallow-worts (*Vincetoxicum* spp.). Environmental Entomology 36(3): 631-636

Christensen T (1998) Swallow-worts. Wildflower, Summer issue: 21-25

Day EH (1882) Proceedings of the Torrey Botanical Club. Bulletin of the Torrey Botanical Club 9: 199-120

Diosdado JC, F Ojeda, J Pastor (1993) CA Stace, Ed. IOPB Chromosome Data 5. Newsletter of the International Organization of Plant Biosystematics 20: 6-7

DiTommaso A, Losey JE (2003) Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. Entomologia Experimentalis et Applicata 108: 205-209

DiTommaso A, Brainard DC, Webster BR (2005a) Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration. Canadian Journal of Botany 83: 102-110

DiTommaso A, Lawlor FM, Darbyshire SJ (2005b) The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [=*Vincetoxicum nigrum* (L.) Moench]. Canadian Journal of Plant Sciences 85: 243-263

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97(13): 7043-7050

Elton CS (1958) The ecology of invasions by animals and plants. University of Chicago Press, Chicago. 181 pp

Ernst CM, Cappuccino N (2005) The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. Biological Invasions 7: 417-425

Gleason HA, Cronquist A (1991) Manual of vascular plants of northeastern United States and adjacent Canada, 2nd Ed. New York Botanical Gardens Press, Bronx, NY. 910 pp

Gray, E (1867) Manual of Botany of the Northern United States. Including the district east of the Mississipi and north of North Carolina and Tennessee, 5th Ed. Ivison, Blakeman, Taylor & Co., New York, NY. 703 pp

Greipsson S, DiTommaso A (2006) Invasive non-native plants alter the occurrence of arbuscular mycorrhizal (AMF) and benefit from that association. Ecological Restoration 24: 236-241

Haeggstrom CA (1990) The influence of sheep and cattle grazing on wooded meadows in Cland, SW Finland. Acta Botanica Fennica 141: 1-28

Hotchkiss EE, DiTommaso A, Brainard DC, Mohler CL (2008) Survival and performance of the invasive vine *Vincetoxicum rossicum* (Apocynaceae) from seeds of different embryony under two light environments. American Journal of Botany (in press)

Inderjit, Callaway RM, Vivanco JM (2006a) Can plant biochemistry contribute to an understanding of invasion ecology? Trends in Plant Science 11(12): 574-580

Inderjit, Weston LA, Duke SO (2006b) Challenges, achievements and opportunities in allelopathy research. Journal of Plant Interactions 1(2): 69-81

Ladd D, Cappuccino N (2005) A field study of seed dispersal and seedling performance in the invasive exotic vine *Vincetoxicum rossicum*. Canadian Journal of Botany 83: 1181-1188

Lauvanger EG, Borgen L (1998) The identity of Vincetoxicum in Norway. Nordic

Journal of Botany 18(3): 353-364

Lawlor FM (2000) Herbicidal treatment of the invasive plant *Cynanchum rossicum* and experimental post control restoration of infested sites. MSc Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY. 126 pp

Lawlor, FM (2002) Element Stewardship Abstract for *Vincetoxicum nigrum* (L.) Moench and *Vincetoxicum rossicum* (Kleopow) Barbarich. http://tncweeds.ucdavis.edu/esadocs/documnts/vinc_sp.pdf . Last accessed 4:32 PM 27/1/08

Lawlor F (2003) The swallow-worts. New York Forest Owner 41(4): 14-15

Lawlor FM, Raynal DJ (2002) Response of swallow-wort to herbicides. Weed Science 50: 179-185

Lee, SK, KA Nam, YH Heo (2003) Cytotoxic activity and G2/M cell cycle arrest mediated by antofine, a phenanthroindolizidine alkaloid isolated from *Cynanchum paniculatum*. Planta Medica 69: 21-25

Liede S (1996) *Cynanchum – Rhodostegiella – Vincetoxicum – Tylophora (Asclepiadaceae):* new considerations on an old problem. Taxon 45: 192-211

Liede S, Tauber A (2002) Circumscription of the genus *Cynanchum* (Apocynaceae – Asclepiadoideae). Systematic Botany 27(4): 789-800

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, Malden (MA). 304 pp

Lumer C, Yost SE (1995) The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York state. Bulletin of the Torrey Botanical Club 122(1): 15-23

Mattila HR, Otis GW (2003) A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog-strangling vine (*Vincetoxicum rossicum*). Entomologia Experimentalis et

Applicata 107: 193-199

Milbrath L, D Gibson (2006) Biological control of swallow-wort and other invasive weeds of the Northeastern United States, 2006 Annual Report. http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO =406908&showpars=true&fy=2006. Last accessed 2:07 PM 27/1/08

Mitchell CE, AA Agrawal, JD Bever, GS Gilbert, RA Hufbaeur, JN Klironomos, JL Maron, WF Morris, IM Parker, AG Power, EW Seabloom, ME Torchin, Dp Vasquez (2006) Biotic interaction and plant invasions. Ecology Letters 9: 726-740

Monachino J (1957) Cynanchum in the New York area. Bulletin of the Torrey Botanical Club 84(1): 47-48

Moore RJ (1959) The dog-strangling vine *Cynanchum medium*, its chromosome number and its occurrence in Canada. Canadian Field Naturalist 73: 144-147

Myers JH, Bazely DR (2003) Ecology and control of introduced plants. Cambridge University Press, Cambridge (UK). 313 pp

Nevo E (1988) Genetic diversity in nature: patterns and theory. Evolutionary Biology 23: 217-246

Nevo E, Beiles A, Ben-Shlomo R (1984) The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. Mani GS, Ed. Evolutionary dynamics of genetic diversity, vol 53. Springer-Verlag, Berlin. Pp 13-213

Nowak, R, W Kisiel (2000) Hancokinol from *Vincetoxicum officinale*. Fitoterapia 71: 584-586

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends in Ecology and Evolution 20(9) DOI 10.1016/j.tree.2005.06.001

Radosevich S, J Holt, C Ghersa (1997) Weed Ecology: Implications for Management

2nd Ed. John Wiley & Sons, Inc., New York, NY. 589 pp

Rejmanek M, RM Richardson (1996) What attributes make some plant species more invasive? Ecology 77(6): 1655-1661

Richards CL, Bossdorf O, Muth N, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9: 981-993

Roberts KJ, Anderson RC (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146: 146-152

Ross MA, CA Lembi (1999) Applied Weed Science, 2nd Ed. Prentice Hall, Upper Saddle River, NJ. 452 pp

Sheeley SE (1992) Life history and distribution of *Vincetoxicum rossicum* (Asclepiadaceae): An exotic plant in North America. MSc Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY. 126 pp

Sheeley SE, Raynal DJ (1996) The distribution and status of species of *Vincetoxicum* in eastern North America. Bulletin of the Torrey Botanical Club 123(2): 148-156

Smith LL (2006) Arbuscular mycorrhizal fungi and the competitiveness of the invasive vine pale swallow-wort (*Vincetoxicum rossicum*). MSc Thesis, Cornell University, Ithaca, NY. 85 pp

Smith LL, DiTommaso A, Lehmann J, Greipsson S (2006) Growth and reproductive potential of the invasive exotic vine *Vincetoxicum rossicum* in northern New York State. Canadian Journal of Botany 84(12): 1771-1780

Smith LL, A DiTommaso, J Lehmann, S Greipsson (2008) Effects of arbuscular mycorrhizal fungi on the exotic invasive vine pale swallow-wort

(*Vincetoxicum rossicum*). Invasive Plant Science and Management (*In press*)

St. Denis M, Cappuccino N (2004) Reproductive biology of *Vincetoxicum rossicum* (Kleo.) Barb. (Asclepiadaceae), an invasive alien in Ontario. Journal of the Torrey Botanical Society 131: 8-15

Staerk, D, J Christensen, E Lemmich, JO Duus, CE Olsen, JW Jaroszewski (2000) Cytotoxic activity of some phenanthroindolizidine *N*-oxide alkaloids from *Cynanchum vincetoxicum*. Journal of Natural Products 63: 1584-1586

Staerk, D, AK Lykkeberg, J Christensen, BA Budnik, F Abe, JW Jaroszewski (2002) In vitro cytotoxic activity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against drug-sensitive and multidrug-resistant cancer cells. Journal of Natural Products 65(9): 1299-1302

Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Bio 4(5): e140 DOI 10.1371/journal.pbio.0040140

Tewksbury L, Casagrande R, Gassmann A (2002) 16. Swallow-worts. Van Driesche R, Lyon RS, Blossey B, Hoddle M, Reardon R, Eds. Biological control of invasive plants in the eastern United States, US Department of Agriculture (USDA) Forestry Service Publication FHTET-2002-04. Washington DC, USDA. Pp 209-216

USDA Plants Database (2008). http://plants.usda.gov Last accessed 4:45 PM 27/1/08

West J, A Fowler (2008) Testimonials. http://www.swallow-wort.com. Last accessed 4:51 PM 27/1/08

Weston LA, Barney JN, DiTommaso A (2005) A review of the biology, ecology and potential management of three important invasive perennials in New York state: japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia*

vulgaris) and pale swallow-wort (*Vincetoxicum rossicum*). Plant and Soil 277(1-2): 53-69

World Meteorological Organization (2008) World Weather Information Service. http://www.worldweather.org. Last accessed 1:43 AM 27/1/2008

Evidence of Allelopathic Activity of the Invasive Species Pale swallow-wort (*Vincetoxicum rossicum*) and Black swallow-wort (*V. nigrum*)

Pale and black swallow-wort (PSW; BSW) are exotic, introduced vines that have rapidly become invasive throughout New York and neighboring regions of Canada and the northeastern US. There are numerous reports in the literature that the two species are allelopathic, allowing them to out-compete and displace native flora in invaded habitats. Results from competition assays in agar-filled magenta boxes demonstrate that swallow-wort root exudates, and particularly those of PSW, are capable of causing significant root length reductions (e.g. 40% for butterfly milkweed) and inhibiting germination rates (e.g. 30% for lettuce) of indicator species. Subsequent bioassays with dried, field-collected tissues demonstrated that leachates from swallow-wort leaf tissues caused an average of 40% and 50% reduction in indicator plant growth from BSW and PSW tissues, respectively. There was a significant dose response, with 1 g of tissues resulting in greater negative effects ($P < 0.05$) than 0.5 g of tissues. Furthermore, tissue leachates of the two swallow-wort species were found to be self-inhibitory. BSW tissues decreased root growth (50%) of PSW indicator plants, while PSW tissues stimulated both root growth (38%) and germination (level: 30%; rate: 43%) of BSW indicator plants. A stimulatory, rather than inhibitory, effect was also observed in some cases for lettuce indicator plants. In a comparative experiment with the related non-invasive native species common milkweed, the two swallow-wort species did not exhibit any significantly greater allelopathic capacity than the milkweed. Although the ecological role for allelopathy in the swallow-worts remains unclear and needs further study, evidence from this study of strong inhibitory activity of swallow-wort tissue leachates has important implications for developing effective management and habitat restoration strategies.

INTRODUCTION

Pale swallow-wort (*Vincetoxicum rossicum* (Kleopow) Babar; PSW) and black swallow-wort (*V. nigrum* (L.) Moench; BSW) are invasive species that were introduced into the northeastern United States over a hundred years ago from the Ukraine and regions of the Iberian Peninsula, respectively (DiTommaso et al. 2005b). While in their native ranges the two vines are relatively rare, the swallow-worts (SWs) have spread rapidly throughout the northeastern US and into southern Canada, typically establishing dense, monocultural thickets (DiTommaso et al. 2005b; Weston et al. 2005). Vegetatively, the two species are nearly identical. The most definitive way to differentiate them is by distinguishing floral features and follicles. Both SWs produce large quantities of seed, many of which are polyembryonic (a condition in which two or more embryos develop from a single egg), although this characteristic is more common in PSW (C. Douglass, Unpublished data). Both species have large root-to-shoot biomass ratios, with Smith (2006) reporting a ratio of 6.7 for some PSW plants in northern New York. While there is some evidence that SW plants can also form stout rhizomes, it is still uncertain to what extent this feature may contribute to the spread of these two species (Cappuccino 2004; DiTommaso et al. 2005b; Lumer and Yost 1995; Weston et al. 2005).

The SWs have historically been used for medicinal purposes in their native ranges, and roots of closely related *Cynanchum* plants are known to contain the poisonous glycoside vincetoxin (Weston et al. 2005). Capo and Saa (1989) reported that (-)-antofine, a phenanthroindolizidine alkaloid isolated from roots of PSW and aerial portions of BSW, had "pronounced" antifungal and antibacterial activity. This class of alkaloid compounds is known to exhibit strong cytotoxic activity (including in cancer cells) and has also been reported as actively inhibiting enzymes involved in the synthesis of DNA and proteins (Lee et al. 2003). More recently, Staerk et al. (2000;

2002) found that several phenanthroindolizidine alkaloids isolated from field-collected PSW plants exhibited marked toxicity against several drug-resistant cancer cell lines. Additionally, Nowak and Kisiel (2000) reported the isolation of the triterpenoid hancokinol from aerial portions of PSW plants, and further stated that this structure was known to possess anti-tumor activity.

Alkaloidal compounds in general are known to have a broad spectrum of biological activity (Rice 1984; Wink and Latz-Bruning 1995). While alkaloids are known to be potent inhibitors of DNA and protein synthesis, their function in interrupting neurotransmissions has led to the suggestion that their role in allelochemical interactions might be merely a side-effect of their primary function as compounds to defend against herbivory (Wink et al. 1999). The role of terpenoid compounds, especially those that are components of essential plant oils, in inhibiting germination and acting as antifungal and antibacterial agents has been well documented (Langheim 1994; Rice 1984).

Cappuccino (2004) was the first to study whether these previously reported chemical compounds in the SWs had any allelopathic effects, and demonstrated that PSW root extracts inhibited germination of radish seeds and showed broad anti-fungal activity. DiTommaso et al. (2005b) suggested that this reported allelopathic activity could indirectly alter mycorrhizal fungi associations that have been determined to be significantly beneficial to the competitive dominance of PSW in particular (also see Greipsson and DiTommaso 2006; Smith 2006; Smith et al. 2008). Furthermore, additional work by N Cappuccino (Unpublished data) has demonstrated that exposure to aqueous extracts of PSW leaf tissues results in potent phytotoxicity. Several authors have proposed that the dense monocultural stands typical of mature SW infestations are, at least, in part due to both the direct exudation of allelochemicals into the rhizosphere and the decomposition of substantial quantities of swallow-wort plant

material (Cappuccino 2004; DiTommaso et al. 2005b; Weston et al. 2005). However, the mechanism of release of allelochemicals by SWs has not been well studied. Moreover, the ground beneath thickets of SW is typically covered with dried stems and leaves of the previous years' growth, and determining whether this layer of degrading tissue serves as a source of leachates with allelochemical properties has not been investigated prior to the research reported here.

The objectives of this study were to determine whether SW root exudates and tissue leachates have allelopathic activity, and investigate the role that allelopathy might play in contributing to the invasiveness of the two species. Specifically, the hypotheses we tested were:

(1) Root exudates of both SW species will decrease growth and inhibit germination of selected juvenile indicator species.

(2) Leachates from dried swallow-wort tissues will reduce growth and inhibit germination in indicator species.

(3) Allelopathic inhibition from SW tissues will be greater than that from common milkweed tissues, a congeneric, non-invasive native species with which SW commonly co-occurs and out-competes in field situations (Smith 2006).

MATERIALS AND METHODS

SW Seed Stratification Protocol. All PSW seeds used in the following experiments were collected at a patchy forest understory site in the Mulholland Wildflower Preserve in Ithaca, NY (42°25'N 76°29'W) in September 2006. BSW seeds were collected at an understory site in Mashomack Nature Preserve at Shelter Island, NY (41°03'N 72°17'W) and in an open field outside the Dutchess County Cornell Cooperative Extension office in Millbrook, NY (41°46'N 73°44'W), both in September 2006. SW seeds were sorted to cull any seeds with visibly abnormal or

enlarged endosperms (after DiTommaso et al. 2005a; Smith 2006). Seeds were placed on three layers of filter paper (Whatman # 1, Whatman Inc, Florham Park, NJ) in the filter compartment of the funnel of a glass vacuum flask, and rinsed for 30 seconds in 100 ml de-ionized water. The seeds were then sterilized for 30 seconds in 100 ml of 50% ethanol, and rinsed again three times, each for 30 seconds, in 100 ml of de-ionized water.

Disks of germination paper (76#, Anchor Paper, St. Paul, MN) were placed in the bottom of round 100 x 20 mm glass Petri dishes. Three milliliters of de-ionized water were added to moisten the paper disk, and up to 65 PSW seeds and 45 BSW seeds placed in each Petri dish. A second disk of germination paper was placed on top of the seeds and moistened with an additional 3 ml of de-ionized water. The Petri dish was sealed with Parafilm and placed in a refrigerator at 1 °C and 20% RH.

Petri dishes were checked every seven days for adequate moisture, with de-ionized water added in 500 μl increments when necessary to ensure that the germination paper disks remained moist. Simultaneously, seeds were checked for fungal growth, and those seeds with prolific fungal contaminations were removed while those with minimal surface infections were washed in fresh de-ionized water and the germination paper disks replaced and re-moistened. Previous work with SW seeds (C. Douglass, Unpublished data) determined that six weeks of cold, moist stratification provided the optimal germination and growth for both SW species, hence all the following experiments were conducted using seeds stratified using the above protocol for six weeks (42 d). Previous studies had also demonstrated that initial seed weight within the range used in our experiments (PSW: 3.9 – 7.9 mg, BSW: 7.7 – 23.9 mg (all dry weights with coma removed)) had no significant correlation with probability or rate of germination, or with early growth rates.

Preparation of Agar. A 0.5% solution (v/v) of Sonneveld nutrient solution (Sonneveld and Stranger 1994) was prepared in de-ionized water. A 1% solution (w/v) of agar gel was created by mixing agar powder (Fisher Bioreagents, Fair Lawn, NJ) into the Sonneveld mixture and heating the solution until the liquid boiled and was clear. A total of 150 ml of this nutrient-enriched agar gel was poured into a Magenta box (Krackeler Scientific Inc, Albany, NY) and autoclaved (Bertin et al. 2003a).

Growth and germination responses to SW root exudates. Five SW seeds of each species were randomly selected from seeds stratified for 42 d. Seeds were placed in a 100 ml glass beaker with1 ml of 50% ethanol and the solution agitated for thirty seconds. Seeds were drained on a metal mesh sieve and rinsed in running de-ionized water for thirty seconds. The surface-sterilized seeds were individually placed on top of the agar gel block in a Magenta box with 5 seeds per box; a seed in each corner of the box and one in the center. These boxes were subsequently placed 5 cm below hanging fluorescent lights (120 V shop lights with Sylvania Cool White Supersaver Ecologic T12 bulbs (34 W), OSRAM Sylvania, Danvers, MA). Ambient conditions were 25 ± 2 °C and 37 ± 3% RH. After five days of growth (a period determined to correspond to at least 75% germination of SW seeds) indicator species were seeded into the Magenta boxes.

Indicator plants were chosen that either grow reliably under controlled laboratory conditions (lettuce (*Lactuca sativa* L.; Black seeded Simpson cv – Johnny's Selected Seeds, Winslow, ME), large crabgrass (*Digitaria sanguinalis* (L.) Scop.; Herbiseed, Twyford, England, UK)) or are species that commonly co-occur with SWs under natural conditions (butterfly milkweed (*Asclepias tuberosa* L.; Everwilde Farms, Bloomer, WI), common milkweed (*Asclepias syriaca* L.; Collected in Ithaca, NY in October 2006) and orchardgrass (*Dactylis glomerata* L.; Stock Seed Farms,

Murdock, NE). Seeds of these five species were sterilized using the surface-sterilization protocol described previously. Seeds were placed on the agar in the Magenta boxes with indicator seeds arranged such that they surrounded the newly germinated SW seeds and were no more than 5 mm away from any one SW seed. Twenty-five seeds of the two monocot indicators (large crabgrass and orchardgrass) and lettuce were added to each Magenta box, while only five seeds of the two milkweed species were used for the experiment due to their large size. The experimental design was a RCBD with trials using lettuce and large crabgrass repeated twice with three replicates each and trials with the remaining indicator species run only once with six replicates per treatment. Variances in number of replicates for indicators was due to time constraints. Treatments in each block were re-randomized every 7 days for the 15-d duration of the experiment.

Growth and germination responses to SW tissue leachates. Experimental methodology used was a modification of the Parker (1966) soil-plant debris assay similar to that used by Weston et al. (1989). Whole PSW plants were collected at the Mulholland Wildflower Preserve in Ithaca, NY (42°25'N 76°29'W) on 19 June 2006. BSW plants were collected in Millbrook, NY on 21 June 2007. Roots, stems, and leaves were separated, then dried at 65 °C for two hours, and 35 °C for 5 days. Dried leaves were placed into a Ziploc plastic bag and hand-crushed until a mixture of fairly evenly sized pieces was obtained. Roots were cut away from the root crown, then placed into a Ziploc plastic bag and hand-crushed until pieces were 10-15 cm in length. Dried stems were cut into 2-cm segments and then ground further using a coffee bean grinder (Cuisinart Grind Central Coffee Grinder, East Windsor, NJ) for 15 to 20 seconds.

Silt loam soil was collected from the Bluegrass Lane Turf and Landscape Research Center (42'22" N 75'22" W) in Ithaca, NY and dried for 12 hours at 80-90 °C. Once cooled the dried soil was sifted through a 2 mm mesh metal sieve. Pure quartz sand was combined with the dried field soil at a 1:1 ratio. A total of 100 g of the soil mixture was placed in the bottom half of a 100 x 15 mm square plastic Petri dish. Either 0.5 or 1 g of one of three types of prepared SW tissue was weighed and spread across the surface of the media. An additional 50 g of the soil mixture was evenly placed on top of the plant tissue and 30 ml of de-ionized water sprayed onto the soil surface. A single piece of filter paper was placed on top of the moistened soil and an additional 5 ml of de-ionized water added to evenly wet the filter paper.

Seeds of indicator species [Annual bluegrass (*Poa annua* L) - Herbiseed, Twyford, England, UK; Barnyardgrass (*Echinochloa crus-gallis* (L.) P. Beauv.) – Valley Seed Service, Fresno, CA; BSW, butterfly & common milkweed, large crabgrass, lettuce, PSW and orchardgrass were from the same sources as above; Tomato (*Solanum lycopersicum* L.) – Red Pear cv, Franchi Sementi S.p.A., Grassobbio Bergamo, Italy] were placed in two rows across the filter paper located 1/3 of the distance inside the edges of the Petri dish. Pairs of monocot and dicot indicator species (monocots: barnyardgrass & large crabgrass; annual bluegrass & orchardgrass; dicots: lettuce & tomato; butterfly & common milkweed; BSW & PSW) were seeded in the same Petri dish. Ten seeds per row were used for all monocots, lettuce and tomato. Five to six seeds per row were routinely used for the dishes containing the other species evaluated due to their larger size. To simulate natural degradation under field conditions, seeds of indicator species for this experiment were not pre-sterilized. SW seeds were stratified for a period of 42 d as described, milkweed seeds were similarly cold stratified for 46 d. Once the seeds were 'planted,' the lids of the Petri dishes were sealed by taping with a 3-4 cm piece of masking tape.

There were twenty-eight treatments for each indicator species. Treatments consisted of combinations of the two SW tissue weights (0.5 and 1 g), three tissue types (root, stem and leaf), and the two SW species. Controls consisted of Petri dishes containing soil and filter paper, but plant tissue was replaced by 0.75 g of shredded, unbleached paper toweling. The experiment was repeated twice for barnyardgrass, large crabgrass, lettuce and tomato with each trial containing three replicates. For the remaining species, the experiment was only run once with each treatment replicated four times. Each trial of the experiment contained a number of blocks equal to the number of treatment replicates, with the order of the Petri dishes containing the indicator seed pairs randomized. Dishes in each block were held together and upright using plastic wrap. An aluminum foil-covered card was placed at the beginning of the set of dishes to minimize any differential light penetration. All dishes and blocks were re-randomized every other day during the 7-d experiment.

Growth and germination response to common milkweed tissue leachates. Entire common milkweed plants were collected in Freeville, NY (42°30'N 76°20'W) on 19 June 2007. Plants were dried according to the same protocol described above except that both root and stem tissues were cut into 2-cm segments prior to drying. Also, dried root and stem segments were first cut into smaller segments and then ground using a rotary blade coffee bean grinder for 30-40 seconds and 15-20 seconds, respectively.

The protocol for this assay is identical to the methodology described above for growth and germination responses to SW tissue leachates except that the indicators used were barnyardgrass, BSW, large crabgrass, lettuce, PSW, and tomato. This experiment was conducted only once using four replicates per treatment.

Statistical Analyses. Prior to analysis of variance (ANOVA), all data were tested for homogeneity of population variances using Levene's test, and equality of variances using plots of residuals and the Shapiro-Wilks test. When the proper assumptions were met, data were run using RCBD ANOVA models. In the case of final germination data for the first experiment, data were arcsine transformed to achieve a normal distribution prior to ANOVA analysis. In some cases it was not possible (due to inherent differences between indicator species in their growth rates and germination) to successfully transform the data. In these cases, Welch test statistics were calculated and reported given the ability for this test to account for the presence of heterogeneous variances as long as population distributions are normal (Clinch and Keselman 1982; Welch 1938; Welch 1947; Zimmerman and Zumbo 1993). Simple t-tests or Tukey HSD tests were used for treatment comparisons in some experiments. The significance of treatment means compared with the control (i.e. 100% or no change) was tested using Dunnet's Method. All statistical analyses were conducted using JMP software (ver. 7 (2007), SAS Institute Inc., Cary, NC).

RESULTS

1. Growth and germination responses to SW root exudates. PSW root exudates significantly reduced root growth of butterfly milkweed (39%, $P < 0.0001$) and large crabgrass (16%, $P < 0.025$), as well as shoot growth of orchardgrass (17%, $P < 0.025$) (**Figure 2.1**). BSW root exudates had a significantly negative effect on shoot growth of common milkweed (16%, $P < 0.05$) only. In contrast, BSW root exudates significantly *stimulated* root growth of lettuce (70%, $P < 0.0001$). Although shoot growth of lettuce was also positively affected (25%) by BSW exudates, and both root (18%) and shoot (14%) growth to a lesser degree from PSW root exudates, these results were not significant.

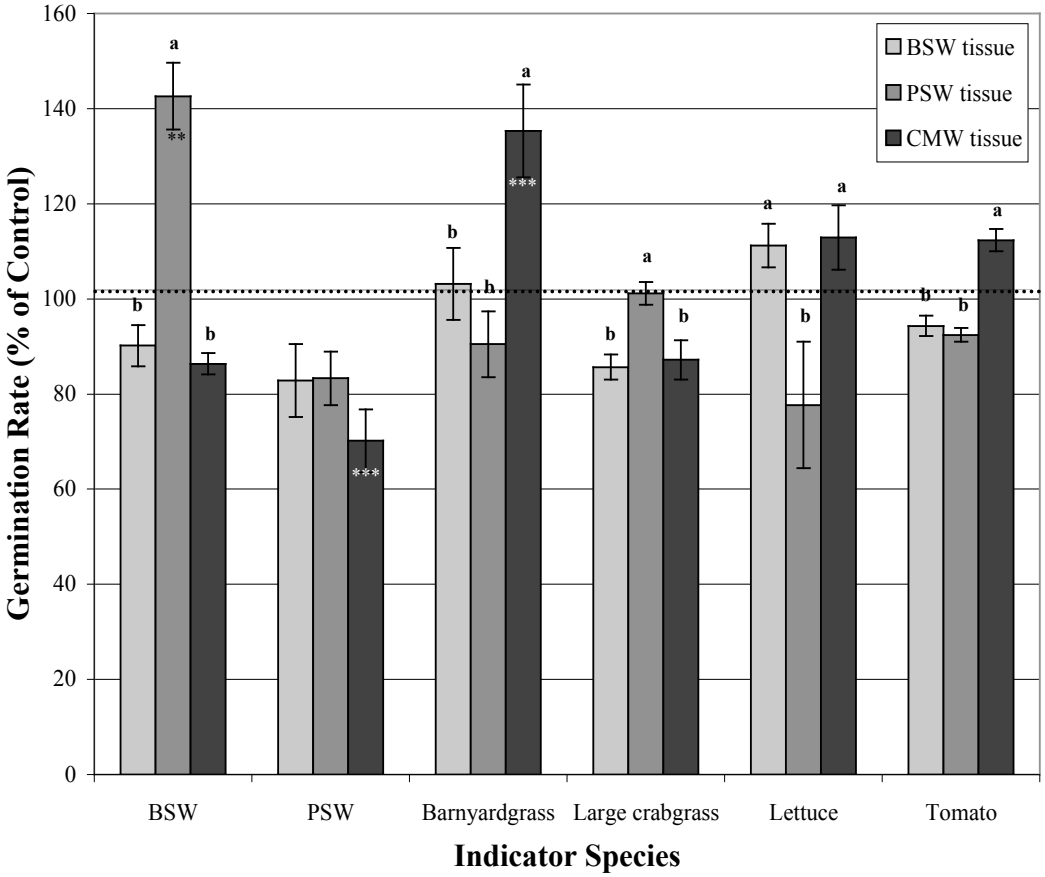


Figure 2.1. Root and shoot length (as % of control) of indicator species (butterfly and common milkweed, large crabgrass, lettuce and orchardgrass) when grown in the presence of SW seedlings for 15 days, in agar-filled Magenta boxes under laboratory conditions. Each value is the mean from two repetitions and three replicates (Magenta boxes) per repetition. Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Lower-case letters above the ± SE bars indicate statistical test for effect of SW species when significant; treatment means sharing the same letter are not significantly different at $P < 0.05$.

SW root exudates did not significantly effect germination of any indicator, except lettuce (**Table 2.1**). BSW root exudates caused a 25% ($P < .0025$) reduction in final lettuce germination and a 37% ($P < 0.0001$) decrease in germination rate of lettuce. PSW exudates resulted in a 10% (NS) reduction in final germination of lettuce, and a 22% ($P < 0.025$) decrease in germination rate.

2. Growth and germination responses to SW tissue leachates.

Root Growth. When data from all indicator species were pooled, there was significantly greater reduction in indicator root growth due to 1 g of SW tissues (pooled across root, stem and leaf tissues) than 0.5 g (**Figure 2.2a**; for data on responses of each indicator see **Appendix I**). One gram of BSW tissues caused a 25% ($P < 0.025$) reduction in the mean root growth of indicator plants, while PSW tissues resulted in a 43% ($P < 0.0001$) decrease in root growth of indicator plants. There was a trend towards a greater effect of PSW tissues compared with BSW on indicator plants. For example, 1 g of PSW tissues caused an 18% ($P < 0.05$) greater negative effect on root growth of indicator species than 1 g of BSW tissues.

Leaf tissues had a greater negative effect than root or stem tissues on the root length of indicator species when data from all indicator plants were pooled (**Figure 2.2b**, data also pooled across 0.5 and 1 g tissue weights). Specifically, leaf tissues of BSW and PSW caused 39% ($P < 0.0001$) and 54% ($P < 0.0001$) reductions in root lengths, respectively. Again, when comparing the effect of leaf tissues from both SW species, PSW tissues caused a 15% ($P < 0.05$) greater negative effect than BSW tissues.

BSW tissues significantly reduced root growth (47%, $P < 0.025$) of PSW indicator plants, and inhibited root growth of BSW indicator plants (8%, NS; **Figure 2.2c**). In contrast, PSW significantly *stimulated* root growth (38%, $P < 0.025$) of

Table 2.1. Final germination and germination rate of indicator species (butterfly and common milkweed, large crabgrass, lettuce and orchardgrass) as affected by PSW and BSW seedlings, from laboratory light bench trials in agar-filled Magenta boxes in which indicators species were grown with SWs for 15 days. Each value is the mean from two repetitions and three replicates (Magenta boxes) per repetition. Absolute values for control (no SW present) are shown at the top of the table, and mean values for the treatments exposed to SW (expressed as percentage of the appropriate control) are below (mean ± SE). ANOVA results[1] - excluding control values - showing probability (P) values for main effects and the primary interaction are also shown. Significance of difference from control is indicated by *, **, *** for $P < 0.05$, 0.025 and 0.0001, respectively. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

Indicator Species	SW Species	Final germination (% seeds)	Germination rate (# seeds/day)
Butterfly milkweed (Control)	None	85.00	1.08
Common milkweed (Control)	None	76.67	1.57
Large crabgrass (Control)	None	94.67	7.07
Lettuce (Control)	None	94.67	6.40
Orchardgrass (Control)	None	75.83	1.59
		Final germination (% of control)	Germination rate (% of control)
Butterfly milkweed	BSW	105. 9	105.6 ab
	PSW	101.0	86.24 ab
Common milkweed	BSW	113.0	103.6 ab
	PSW	100.0	91.69 ab
Large crabgrass	BSW	99.23	94.58 ab
	PSW	102.1	102.1 ab
Lettuce	BSW	**75.12 ****	**62.81 b *****
	PSW	89.50	**78.41 b ****
Orchardgrass	BSW	91.21	90.09 ab
	PSW	118.7	127.7 a
ANOVA (P) Values[1]			
Indicator Species (I)		**0.0391** †	**0.0016** †
SW Species (SW)		0.3958	0.4278
Indicator Sp. (I) X SW Sp. (SW)		**0.0378** †	**< 0.0001** †

1. Where indicated by the symbol '†' the populations used in the ANOVA comparison did not satisfy the assumption of homogeneity of variances, and so the value given in the table is that of the Welch's ANOVA, which corrects for the presence of heterogenous population variances.

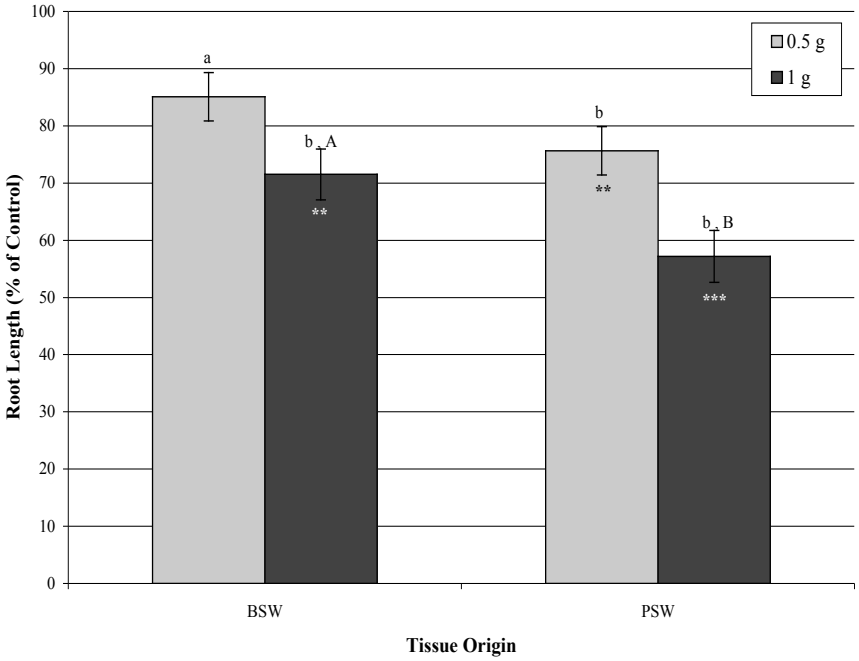


Figure 2.2a. Root length (as % of control) of pooled indicator species when grown in the presence of leachates from dried, field-collected SW tissues for 7 days, from modified Parker bio-assays under laboratory conditions. Vertical bars represent ± SE. Significance of difference from control (100 %) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Results are pooled across the three tissue types (leaf, root, and stem); each value is the mean from two repetitions and 180 replicates. Lower-case letters above the SE bars indicate statistical test for effect of tissue weight when significant, upper-case letters test for effect of SW species (tissue origin) when significant. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

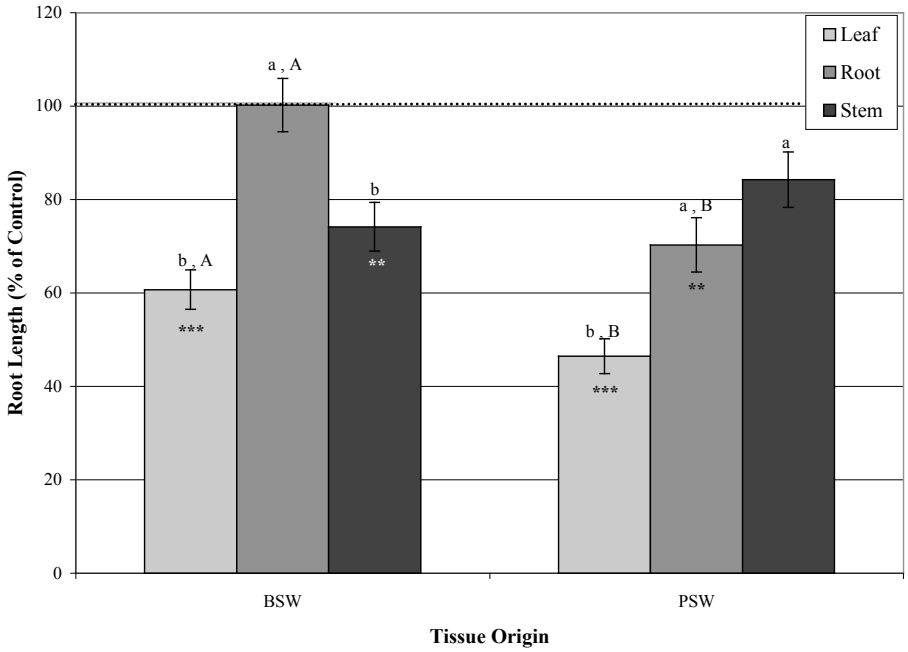


Figure 2.2b. Root length (as % of control) of pooled indicator species when grown in the presence of leachates from dried, field-collected SW tissues for 7 days, from modified Parker bio-assays under laboratory conditions. Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Results are pooled across the two tissue weights (0.5 and 1 g); each value is the mean from two repetitions and 120 replicates. Lower-case letters above the SE bars indicate statistical test for effect of tissue type (leaf, root and stem) when significant, upper-case letters test for effect of SW species (tissue origin) when significant. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

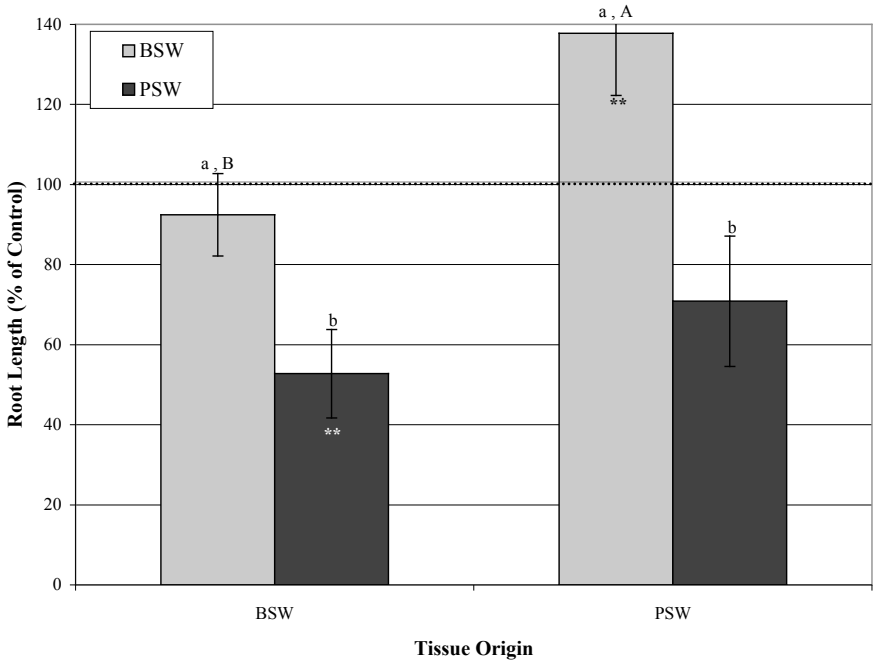


Figure 2.2c. Root length (as % of control) of SW species when grown in the presence of leachates from dried, field-collected SW tissues for 7 days, from modified Parker bio-assays under laboratory conditions. Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Results are pooled across the two tissue weights (0.5 and 1 g) and three tissue types (leaf, root and stem); each value is the mean from two repetitions and 36 (SW indicator plants) replicates. Lower-case letters above the ± SE bars indicate statistical test for significantly different response of SW species, upper-case letters test for effect of SW species (tissue origin) when significant. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

BSW indicator plants, while inhibiting the root growth of PSW indicator plants (29%, NS).

Germination. SW tissue effects on final seed germination and germination rate of indicator plants contrasted markedly with their effects on growth of indicator species (for data on responses of each indicator see **Appendix I**). For example, there were no significant differences in the effects of the two tissue weight treatments or three tissue types on seed germination of indicator species.

The only significant effects of SW tissues on germination were the effects on SW species. PSW tissues resulted in a 30% ($P < 0.025$) *stimulation* in final germination of BSW indicator plant seeds and a 33% ($P < 0.025$) inhibition in the germination of PSW seeds (**Figure 2.3a**). BSW tissues had no effect on the germination rate of the two SW species, but PSW tissues *stimulated* the rate of germination in BSW indicator plant seeds by 43% ($P < 0.025$) and inhibited the germination rate of PSW seeds (17%, NS; **Figure 2.3b**).

3. Growth and germination responses to common milkweed tissue leachates. All three tissue sources (BSW, PSW and common milkweed) reduced root length of PSW and large crabgrass indicator plants significantly, with mean reductions of 38% ($P < 0.025$) and 41% ($P < 0.0001$), respectively (**Figure 2.4a**). BSW root length was also inhibited by common milkweed tissues (38%, $P < 0.0001$). Conversely, common milkweed tissues resulted in a 122% ($P < 0.0001$) stimulation of root length in lettuce. Shoot length was reduced in only a single indicator species, large crabgrass, by common milkweed tissues (25%, $P < 0.025$; **Figure 2.4b**).

BSW, PSW and common milkweed tissues caused similarly significant decreases (mean = 23%, $P < 0.05$) in the final number of germinated PSW indicator

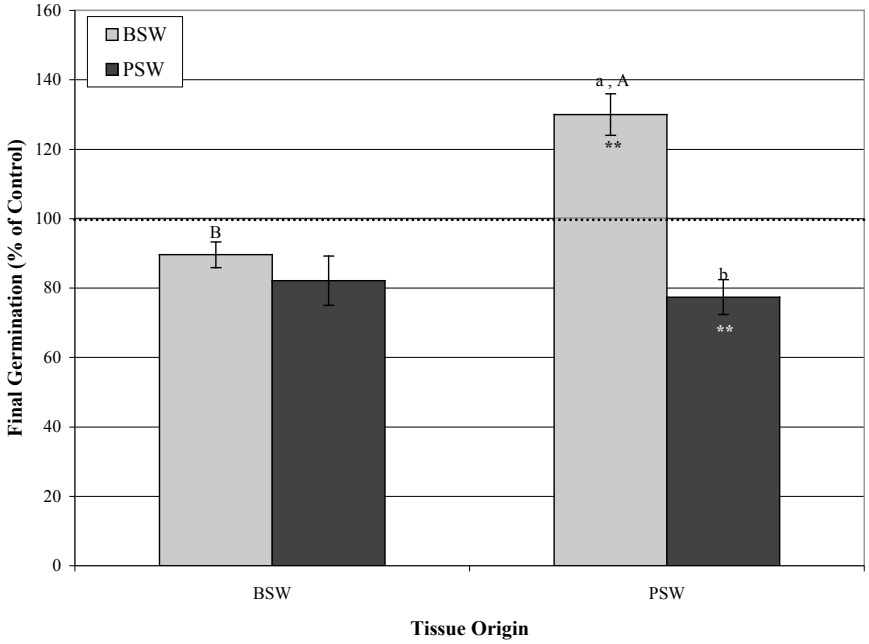


Figure 2.3a. Final germination (as % of control) of SW species when grown in the presence of leachates from dried, field-collected SW tissues for 7 days, from modified Parker bio-assays under laboratory conditions. Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Results are pooled across the two tissue weights (0.5 and 1 g) and three tissue types (leaf, root and stem); each value is the mean from two repetitions and 36 (SW indicators) replicates. Lower-case letters above the ± SE bars indicate statistical test for significantly different response of SW species, upper-case letters test for effect of tissue origin (BSW or PSW) when significant. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

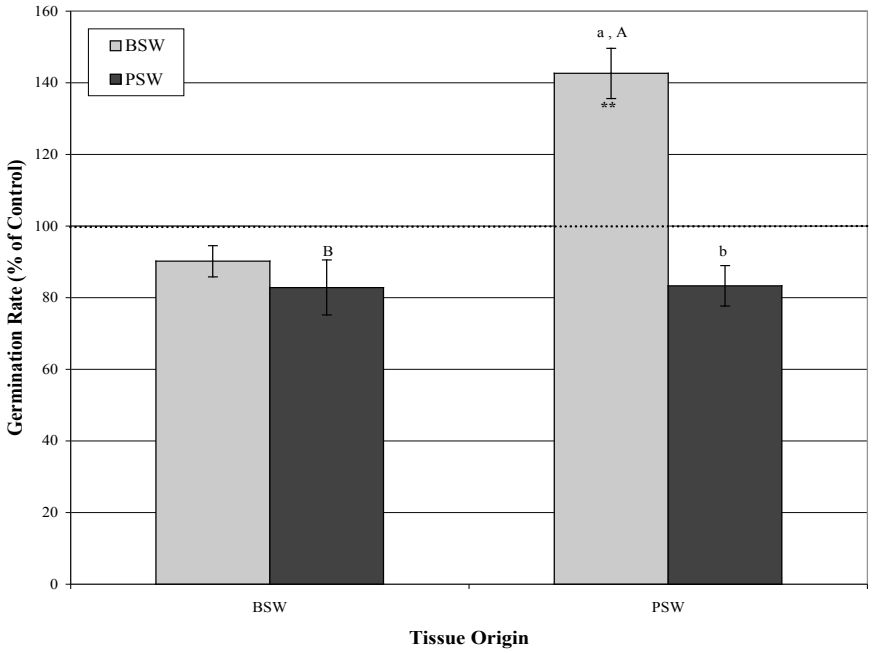


Figure 2.3b. Germination rate (as % of control) of SW species when grown in the presence of leachates from dried, field-collected SW tissues for 7 days, from modified Parker bio-assays under laboratory conditions. Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, **, *** below the bars for $P < 0.05$, 0.025, and 0.001, respectively. Results are pooled across the two tissue weights (0.5 and 1 g) and three tissue types (leaf, root and stem); each value is the mean from two repetitions and 36 (SW indicators) replicates. Lower-case letters above the ± SE bars indicate statistical test for significantly different response of SW species, upper-case letters test for effect of tissue origin (BSW or PSW) when significant. Treatment means sharing the same letter are not significantly different at $P < 0.05$.

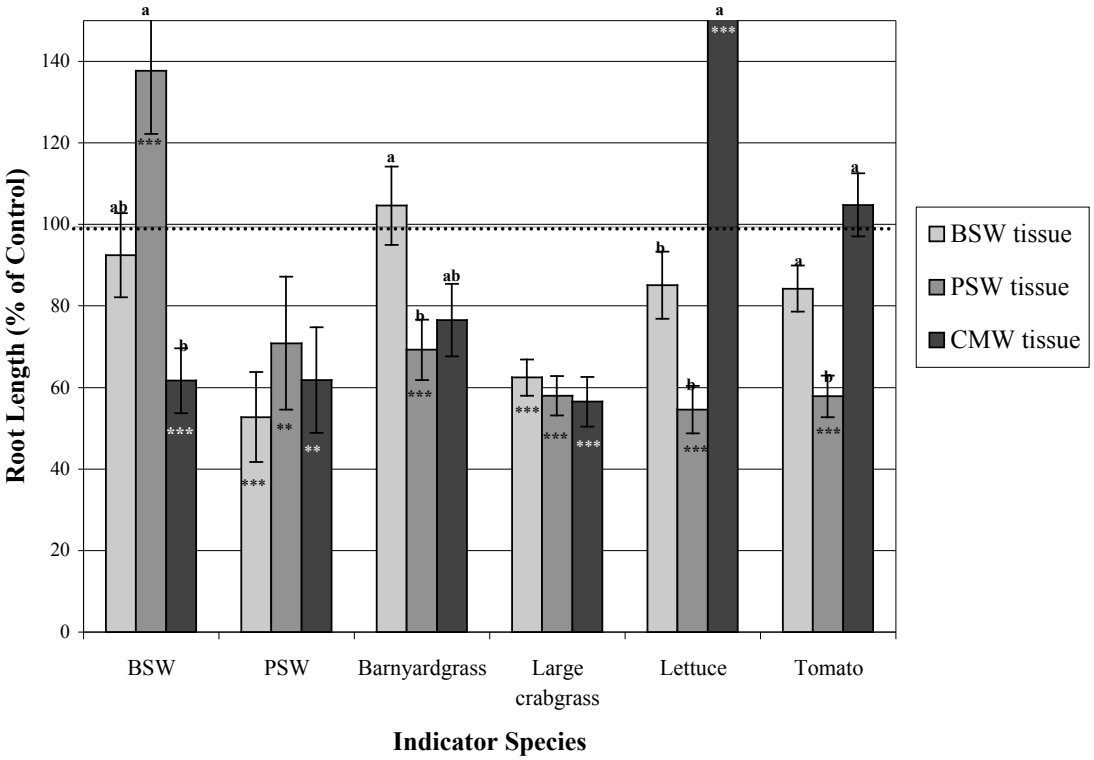


Figure 2.4a. Root length (as % of control) of indicator species when grown in the presence of leachates from dried, field-collected SW and common milkweed (CMW) tissues. Data is pooled across three tissue types (root, shoot and leaf) and two tissue weights (0.5 g and 1 g) for all three tissue origins (see Appendix I for individual treatment responses). Results from modified Parker bio-assays conducted under laboratory conditions in Petri dishes. Each value is the mean from two repetitions and three replicates (Petri dish). Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, ** and *** below the bars for $P < 0.05$, 0.025 and 0.001, respectively. Lower-case letters above the ± SE bars indicate statistical test for effect of tissue origin (BSW, PSW or common milkweed) when significant; treatment means sharing the same letter are not significantly different at $P < 0.05$.

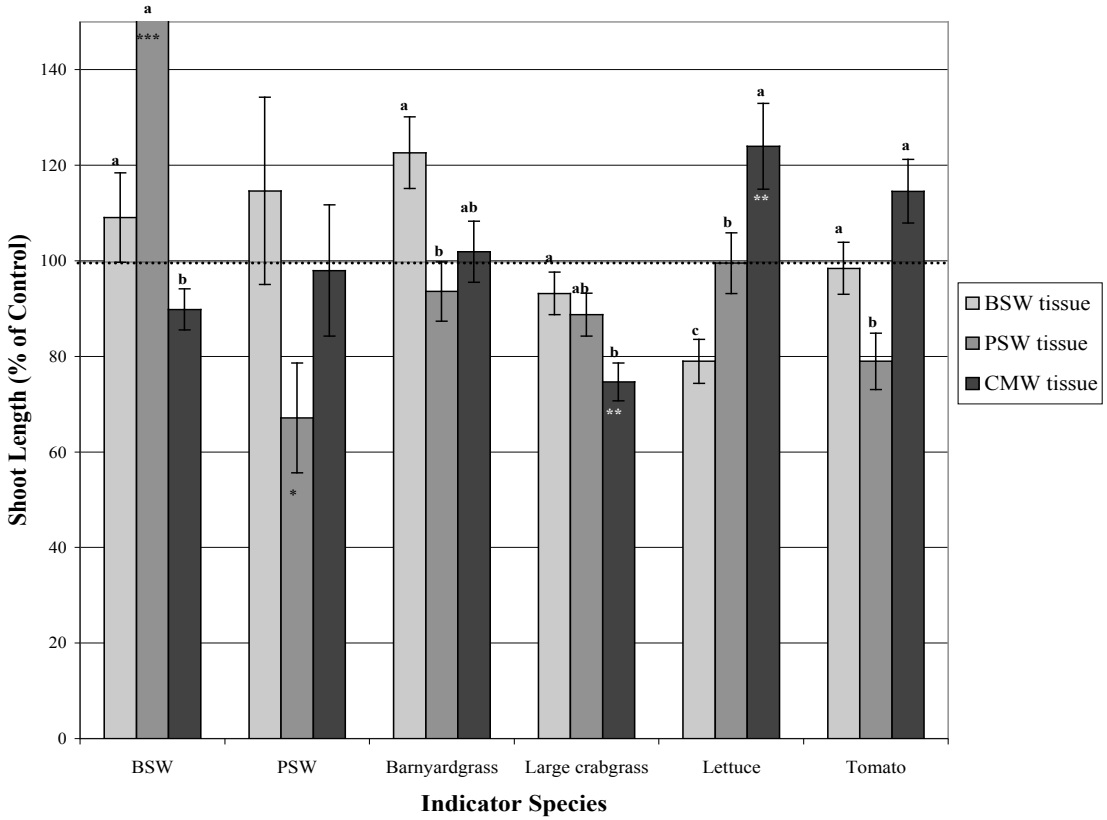


Figure 2.4b. Shoot length (as % of control) of indicator species when grown in the presence of leachates from dried, field-collected SW and common milkweed tissues. Data is pooled across three tissue types (root, shoot and leaf) and two tissue weights (0.5 g and 1 g) for all three tissue origins (see Appendix I for individual treatment responses). Results from modified Parker bio-assays conducted under laboratory conditions in Petri dishes. Each value is the mean from two repetitions and three replicates (Petri dish). Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, ** and *** below the bars for $P < 0.05$, 0.025 and 0.001, respectively. Lower-case letters above the ± SE bars indicate statistical test for effect of tissue origin (BSW, PSW or common milkweed) when significant; treatment means sharing the same letter are not significantly different at $P < 0.05$.

plant seeds (**Figure 2.5a**). Large crabgrass germination was not affected by either SW species, but was reduced 18% ($P < 0.025$) by common milkweed. Similarly, the germination rate of PSW indicators was significantly inhibited by common milkweed leachates (30%, $P < 0.0001$). In contrast, final germination and germination rate of barnyardgrass indicator seeds were stimulated by common milkweed residues by 23% ($P < 0.0001$) and 35% ($P < 0.0001$), respectively (**Figures 2.5a** and **2.5b**).

DISCUSSION

Evidence of inhibitory effects from BSW and PSW, particularly on root growth of indicator species, was documented in separate experiments that examined effects from SW root exudates (**Figure 2.1**), and effects from leachates of field-collected SW tissues (especially leaf tissues and at higher application weights (1g); **Table 2.2**). Shoot growth and seed germination were also inhibited in some cases, but not as consistently, and usually the effect was smaller in magnitude than effects on roots. Radical elongation and root growth are often more sensitive to the phytotoxic properties of suspected allelochemicals than shoot growth or seed germination (Bertin et al. 2003a; Stowe 1979).

There were differences in sensitivity of the various indicator species used, and in some cases the two SW species stimulated rather than inhibited growth. In our evaluation of responses to leachates from field-collected SW plants, large crabgrass was most consistently negatively affected (primarily root and shoot growth) by both BSW and PSW (**Table 2.2**). Lettuce and tomato were also sensitive (**Table 2.2**).

Variation in the responses of indicator species to suspected allelopathic exudates or tissue leachates is commonly reported in the literature (Newman and Rovira 1975; Orr et al. 2005; Stowe 1979). Schulz and Friebe (1999) observed that differential responses by indicator species to allelochemicals are often due to

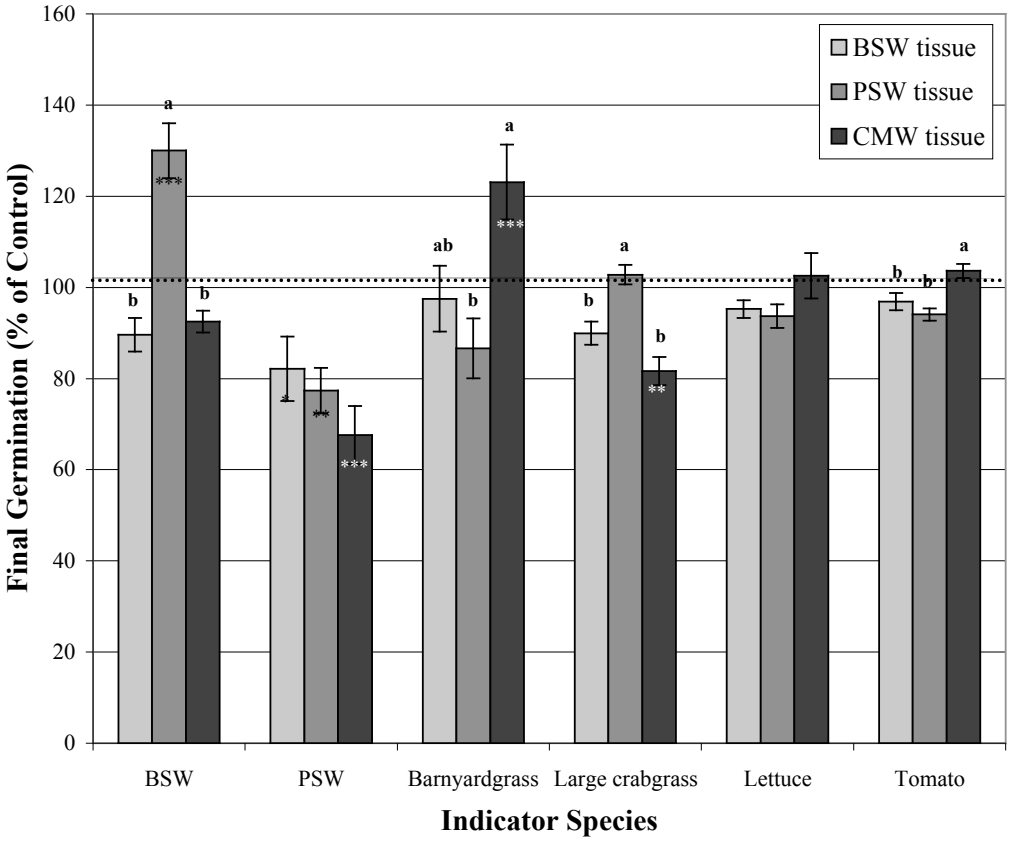


Figure 2.5a. Final germination (as % of control) of indicator species when grown in the presence of leachates from dried, field-collected SW and common milkweed tissues. Data is pooled across three tissue types (root, shoot and leaf) and two tissue weights (0.5 g and 1 g) for all three tissue origins (see Appendix I for individual treatment responses). Results from modified Parker bio-assays conducted under laboratory conditions in Petri dishes. Each value is the mean from two repetitions and three replicates (Petri dish). Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, ** and *** below the bars for $P < 0.05$, 0.025 and 0.001, respectively. Lower-case letters above the ± SE bars indicate statistical test for effect of tissue origin (BSW, PSW or common milkweed) when significant; treatment means sharing the same letter are not significantly different at $P < 0.05$.

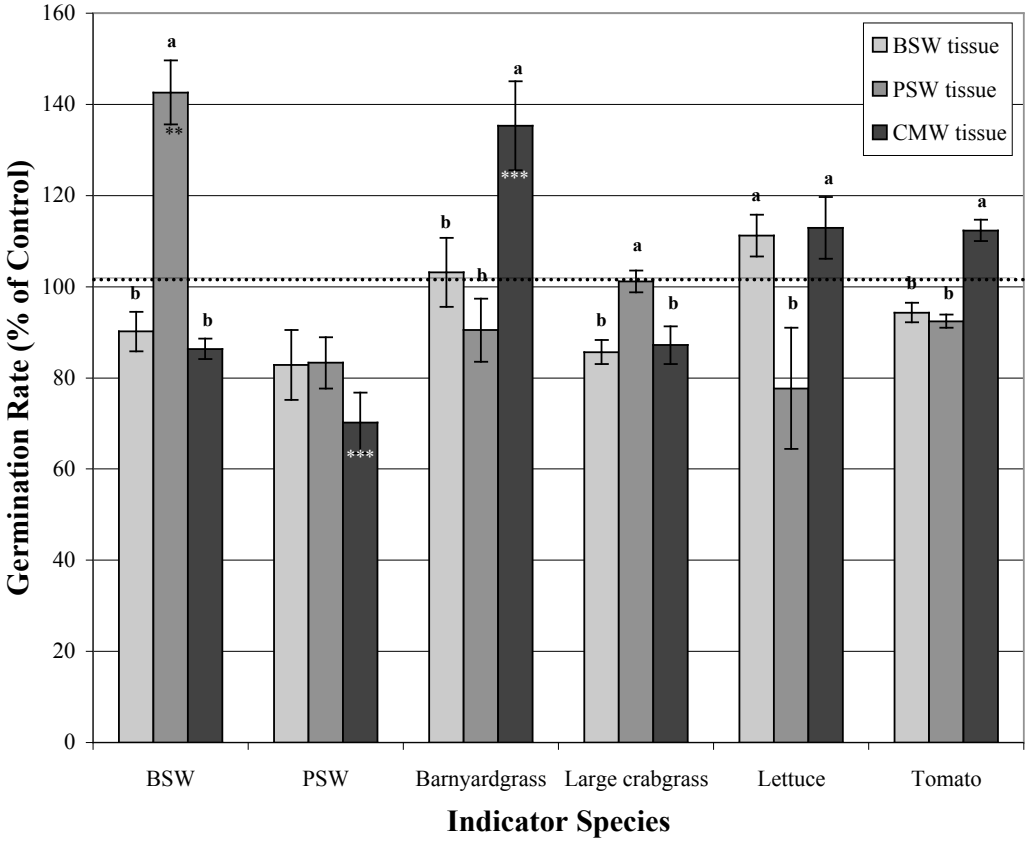


Figure 2.5b. Germination rate (as % of control) of indicator species when grown in the presence of leachates from dried, field-collected SW and common milkweed tissues. Data is pooled across three tissue types (root, shoot and leaf) and two tissue weights (0.5 g and 1 g) for all three tissue origins (see Appendix I for individual treatment responses). Results from modified Parker bio-assays conducted under laboratory conditions in Petri dishes. Each value is the mean from two repetitions and three replicates (Petri dish). Vertical bars represent ± SE. Significance of difference from control (100 % - dotted line) is indicated by *, ** and *** below the bars for $P < 0.05$, 0.025 and 0.001, respectively. Lower-case letters above the ± SE bars indicate statistical test for effect of tissue origin (BSW, PSW or common milkweed) when significant; treatment means sharing the same letter are not significantly different at $P < 0.05$.

Table 2.2. Summary of the effects of SW (BSW & PSW) and common milkweed (CMW) tissues (pooled across three tissue types (root, shoot and leaf) and two tissue weights (0.5 g & 1 g)) on indicator species. Measures of effect are: root growth (R); shoot growth (Sh); final germination (FG); and germination rate (GR). Significant ($P < .05$) stimulations in any measure is indicated by '+,' significant ($P < .05$) reductions by '-', and no significant effect by '0.' See **Figures 2.4a** (root growth), **2.4b** (shoot growth), **2.5a** (final germination), **2.5b** (germination rate), or **Appendix I** (raw data for all treatments) for additional data.

Tissue Origin	Indicator Species																							
	BSW				PSW				Barnyardgrass				Large Crabgrass				Lettuce				Tomato			
	R	Sh	FG	GR	R	Sh	FG	GR	R	Sh	FG	GR	R	Sh	FG	GR	R	Sh	FG	GR	R	Sh	FG	GR
BSW	0	0	0	0	-	0	-	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0
PSW	+	+	+	+	-	-	-	0	-	0	0	0	-	0	0	0	-	0	0	0	-	0	0	0
CMW	-	0	0	0	-	0	-	-	0	0	+	+	-	-	-	0	+	+	0	0	0	0	0	0

tolerances for and/or detoxification of the biologically-active compounds by specific indicator plants. PSW reduced root growth of indicator species more substantially than BSW (**Figures 2.2a** and **2.2b**). In particular, with 1 g of leaf tissues PSW always caused greater decreases in root growth than BSW tissues. Few studies have compared the relative allelopathic effects of closely related congeneric species. In the case of knapweed (*Centaurea* spp.) invasion in the western US, spotted (*C. maculosa* Lam.) and diffuse knapweed (*C. diffusa* Lam.) differ in allelochemicals and mode of activity (Bais et al. 2003; Callaway and Aschehoug 2000; Inderjit et al. 2006).

BSW tissues reduced root growth in PSW indicator plants by 50%, but caused no change in growth of BSW indicator plants (**Figure 2.2c**). In contrast, PSW tissues stimulated both root growth and seed germination of BSW plants by 30 to 43%. Given that the introduced ranges of the two species were until recently distinct, and are now beginning to overlap, this finding of a BSW advantage from allelopathic interference between these two congeneric invasive species is ecologically important. In addition to the outcome associated with competition between the two species, our results suggest that allelopathy may also play a key role in relative invasion success of the congeneric species, at least in terms of initial seedling establishment.

In addition, PSW tissues reduced the growth of PSW indicator plants by 23% (**Figure 2.2c**). This finding could be due to autotoxicity, which has been reported to occur with competitively dominant species as a means of limiting the effects of intra-specific competition (Fitter and Hay 1987; Newman and Rovira 1975).

Stimulation in Growth and Germination of Indicator Species. In SW root exudate bioassays root and shoot growth of lettuce indicator plants were stimulated by the presence of both SW species (**Figure 2.1**). When results for tissue leachate bioassays were pooled (by tissue weight, origin, and type), shoot length in BSW and butterfly

milkweed plants were stimulated (**Appendix I**). When indicator species results were pooled the 0.5 g treatment weight (results averaged by tissue origin and type), there was a stimulation in shoot growth (17%, P = 0.13), but no effect was observed in the 1 g of tissue weight treatment (**Figure 2.4b**).

Similarly, Stevens and Merrill (1985) found that low concentrations (10 PPM) of several sesquiterpene lactones stimulated root elongation in lettuce (cv. Black seeded Simpson) but that higher concentrations inhibited growth. Rice (1986) also reported strong stimulation of growth in both downy brome (*Bromus tectorum* L.) and radish (*Raphanus sativus* L.) seedlings by both decaying leaves and root exudates of ground ivy (*Glechoma hederacea* L.). Orr et al. (2005) found that leaf tissue leachates of tall fescue (*Festuca arundinaceum* (Schreb.) S.J. Darbyshire) and autumn olive (*Elaeagnus umbellata* Thunb.) stimulated seed germination and survival of eastern cottonwood (*Populus deltoids* Bartram ex Marsh.). The authors hypothesized that this positive effect was likely due to the release of nutrients or hormones by the decaying plant tissues that stimulated germination of cottonwood seeds and enhanced seedling survival.

The ability of leachates and exudates to stimulate the growth of some indicator species does not discount that SWs may be allelopathic. Specifically, certain SW tissues may release nutrients or stimulators of seed germination and seedling growth at different rates over time, and seedling indicator species may exhibit differences in response to these tissue exudates. Indeed, increases in shoot growth could be considered a natural response to decreased nutrient allocation in roots due to toxic effects in these structures (Fitter and Hay 1987; Nilsson 1994). However, from our mixed findings as to the effect direction (+/-) of SW tissues, it is difficult to interpret how much of a competitive advantage allelopathy might provide to SW seedlings, given the stimulatory effect observed in seedlings of potentially competing species.

Nonetheless, given that newly established SW seedlings exhibited significant inhibitory effects on some indicator species tested, the impact of well-established SW populations on neighboring vegetation is worthy of further examination in field conditions.

Invasiveness and enhanced interference by SW species. A comparison of the effects of PSW and common milkweed tissues resulted in an equivalent degree of biological activity among the two species, but a lower level of activity was found with BSW tissues (**Table 2.2**). Allelopathy in milkweed species (Asclepiadaceae) has been little studied, but yield reductions in sorghum (*Sorghum bicolor* (L.) Moench) and decreases in growth and seed germination of wheat (*Triticum aestivum* L.) varieties due to exudates and aqueous extracts of aerial portions of common milkweed plants have been reported (Rice 1984). It would be interesting to determine if similar allelochemicals are present in both PSW and BSW tissues compared with the distantly related common milkweed.

The strong allelopathic activity reported in this study of both PSW and common milkweed is valuable in the context of research indicating that under natural field conditions, common milkweed is frequently out-competed by PSW (DiTommaso and Losey 2003; Mattila and Otis 2003). Our results suggest that direct interference by allelopathy may not provide a strong advantage for invasive SWs in interactions with common milkweed. Smith (2006) found that the manipulation of soil microbial communities by *V. rossicum* provided this species a competitive advantage in root-to-shoot biomass production over common milkweed (also Greipsson and DiTommaso 2002; Smith et al. 2008).

Ecological Context. The variable responses obtained for specific indicator species in this study suggest that direct allelopathic activity of root exudates by SW seedlings may not play a strong ecological role in enhancing the ultimate competitive ability of invasive SW species. Rather than direct allelopathic toxicity, we suggest that SW plants primarily benefit from the biological activity of allelochemicals indirectly by the potential impact of exudates and/or leachates on soil microbes or fungal species, which in turn ultimately benefit the invasive species (Inderjit and Weiner 2001; Kruse et al. 2000; Mitchell et al. 2006; Wardle et al. 1998; Wolfe and Klironomos 2005). Alternatively, allelochemicals released from SWs could alter nutrient cycling and availabilities in the rhizosphere, which can result in changes in the soil microflora (Facelli and Pickett 1991; Hodge and Millard 1998; Inderjit and Weston 2003).

The occurrence of instances of growth stimulation in our bioassays are important to consider in this context as plant root exudates and decomposing aboveground residues can play a significant role in the stimulation of the growth of fungal and bacterial communities in the soil (Bertin et al. 2003b; Facelli and Pickett 1991; Inderjit and Weston 2003; Rice 1986). Specifically, Blum (1995) found that the addition of phenolic acids to soils resulted in the stimulation or selection for microbial species that were able to utilize these compounds as carbon sources. Although these soil rhizosphere interactions are little studied, further impacts of SW establishment upon soil microbial dynamics would be interesting, given the amount of root biomass produced especially by PSW in established communities.

Research conducted in New York has demonstrated that PSW plants can substantially alter soil microbial communities in invaded habitats by facilitating the growth of beneficial species of mycorrhizal fungi. This change in the microbial community may result in the displacement of native plant species that require specific mycorrhizal (or other fungal) species for growth (DiTommaso et al. 2005b; Greipsson

and DiTommaso 2006; Smith 2006; Smith et al. 2008). These authors were unable to determine a specific mechanism by which the alteration of microbial communities could occur, and given the strong responses in indicators caused by putative allelochemicals in both SW species we suggest that this process could be mediated via allelopathic root exudates or SW tissue leachates. The role that these biologically active root exudates and leachates play in rhizosphere interactions within invaded habitats could be similar to that found in garlic mustard *(Alliaria petiolata* (M. Bieb.) Cavara & Grande) infested areas. In these invaded habitats, root exudates of garlic mustard have been shown to suppress the growth of arbuscular mycorrhizal fungi (AMF) species whose presence is required for the growth and success of native tree seedlings (Roberts and Anderson 2001; Stinson et al. 2006).

Management implications. Leachates from SW tissues resulted in greater reductions in root growth and seed germination of indicator species than SW root exudates. Root exudates (results pooled across the two SW species) caused two cases (of the five indicator species) of significant root length reduction and a single case in which shoot length was reduced (**Figure 2.1**). Comparatively, tissues leachates (effects averaged across tissue weight, origin, and type) resulted in 7 indicator species (of the 10) with significant root length decreases and for several species germination was inhibited (**Figures 2.4a, 2.4b, 2.5a** and **2.5b**). The low activity of root exudates compared to tissue leachates in our bioassays may be due to a reduction in the production of bioactive secondary products in root tissues in comparison to shoot tissues, or simply differences in allelochemical constituents (Rice 1984). Given that the production of root exudates and their inhibitory activity would increase with greater root biomass of mature SW plants under field conditions, more research is needed (Smith 2006; Weidenhamer 1996).

One gram compared to 0.5 g of SW tissues (pooled across tissue origin, type, and indicator species) caused a two-fold increase in growth reductions and also increased reductions in several germination parameters, in contrast lower tissue weights stimulated shoot growth (**Appendix I**). This dose response effect suggests that allelopathic activity by SWs requires a threshold account of bioactive compounds (Nilsson 1994).

Of the three tissue types, leaf tissues were most active while stem tissues were moderately active. Leaf tissues (when pooled by tissue weight, origin, and indicator species) decreased root length in indicator species by half, while stem tissues caused a 21% decrease in root growth (**Figure 2.2b**). The increased activity of leachates from aerial portions of SW plants is not surprising given the presence of biologically-active chemistries reported in leaf and stem tissues specifically (Capo and Saa 1989; Nowak and Kisiel 2000; Staerk et al. 2000 & 2002). Numerous authors have found that leachates from shoot or leaf tissues caused greater reductions in indicator species growth than undergound plant structures (Orr et al. 2005; Weston and Putnam 1986). Specifically, Wardle et al. (1996) found that decomposing shoot tissues of several common New Zealand forage grasses strongly reduced radicle elongation of *Carduus nutans* L. seedlings, while root tissues of these grasses stimulated shoot production in this indicator species.

From a management perspective, these findings suggest that there could be unexpected ramifications of what is currently one of the recommended control strategies for the two species – mowing. This method of control has been found to be effective at preventing populations from spreading if timed to coincide with seed set and repeated throughout the growing season (DiTommaso et al. 2005b; Lawlor 2000; McKague and Cappuccino 2005; Sheeley 1992). Our research suggests that the abundant SW leaf and stem material remaining on the soil surface after mowing or

defoliation could potentially release allelochemicals into the soil rhizosphere, resulting in inhibition of seed germination and seedling growth of sensitive plant species.

Allelopathic effects of leachates from decomposing crop residues in agricultural systems has been well documented (Altieri 1995; Kruse et al. 2000; Putnam and Weston 1986; Radosevich et al. 1997; Rice 1984). While our current understanding of which specific chemicals in SW leachates (or root exudates) are responsible for the allelopathic activity observed in our experiments is limited, if the biologically active components of tissue leachates were persistent in the soil they could prevent the germination and establishment of favorable species during restoration of the affected sites. Mitigating the environmental legacies of changes in ecosystem processes caused by exotic invasive plants, e.g. alterations in soil salinity and nutrient levels, should be taken into account when planning and implementing restoration strategies (D'Antonio and Meyerson 2002; Gordon 1998; Renne et al. 2005; Van Andel and Aronson 2005).

Summary. We found clear evidence of inhibitory effects of SW plant tissues and leachates on indicator plants, particularly root growth, but also shoot growth and germination parameters. Effects due to leachates of leaf tissues, and at higher tissue weights were greater than those from root exudates of young seedlings. The magnitude of negative effects on growth and germination varied with indicator species and specific experimental protocols, but in general tissue leachates and exudates from PSW caused greater inhibitory activity than those from BSW.

Leachates from PSW tissues stimulated growth of BSW, while BSW tissue leachates inhibited or had no effect on PSW in our experiments. Due to stimulatory effects of SWs on one indicator species (lettuce), and the finding that leachates of common milkweed tissues resulted in equivalent inhibition of selected indicators, we

cannot conclude that allelopathic activity of SW species plays a dominant role in determining SW invasiveness and competitiveness.

APPENDIX I

Table A1a. Root and shoot length (as % of control) of indicator species in response to 0.5 g of dried tissues (root, stem and leaf) collected from field stands of PSW and BSW plants. Indicator seeds were grown for 7 days in modified Parker bio-assays conducted under laboratory conditions. Absolute values for control (no swallow-wort tissue present) are shown in the left-hand column, followed by values for treatments exposed to swallow-wort tissues. Each value is the mean from two repetitions and three replicates (Petri dishes) per repetition. Significance of difference from control is indicated by *, **, *** for P < 0.1, 0.05, and 0.001, respectively. Where 0.1 < P < 0.05 the actual value of significance is given; coefficient of variation (CV) for root data = 65.38, for shoot data = 55.63.

Root Length (% of control)							
Indicator Species	Control (mm)	Root tissues		Stem tissues		Leaf tissues	
		BSW	*PSW*	*BSW*	*PSW*	*BSW*	*PSW*
Annual bluegrass	5.0	171.5	46.0	87.1	77.1	49.7	48.3
Barnyardgrass	19.9	116.1	43.5	74.4	91.1	97.6	87.0
Black swallow-wort	22.9	121.7	181.3	106.6	164.8	47.0	74.1
Butterfly milkweed	10.0	187.6 ***	90.52	72.5	80.3	138.8	45.7 * (0.07)
Common milkweed	18.8	79.8	64.8	73.0	92.0	94.0	30.9
Large crabgrass	16.4	89.4	62.71	51.0	69.4	78.7	68.8
Lettuce	17.8	127.9	46.4	73.5	63.1	69.1	64.9
Orchardgrass	27.6	58.6	70.2	53.6	125.9	43.3	86.2
Pale swallow-wort	9.2	74.1	142.0	39.2	103.0	35.2	31.9
Tomato	31.9	114.0	60.4	104.2	85.4	78.8	40.9
Shoot Length (% of control)							
Annual bluegrass	3.8	208.4	81.6	108.1	126.6	139.6	11.3
Barnyardgrass	18.8	115.4	54.2	99.1	98.9	154.3	111.0
Black swallow-wort	11.5	128.9	232.8	104.5	231.8	83.9	155.4
Butterfly milkweed	11.3	199.2	147.2	128.6	210.6	240.7	171.1
Common milkweed	17.7	144.1	119.2	129.2	142.1	208.4	68.3
Large crabgrass	20.5	106.8	86.2	96.9	107.8	111.4	80.8
Lettuce	16.8	92.4	99.3	79.6	119.7	72.6	98.0
Orchardgrass	16.1	63.0	111.1	90.0	116.0	77.7	186.9
Pale swallow-wort	8.5	92.5	100.3	139.4	102.6	156.7	95.6
Tomato	24.0	110.3	75.7	124.9	110.8	94.6	58.0

Table A1b. Root and shoot length (as % of control) of indicator species in response to 1 g of dried tissues (root, stem and leaf) collected from field stands of pale (PSW) and black swallow-wort (BSW) plants. Indicator seeds were grown for 7 days in modified Parker bio-assays conducted under laboratory conditions. Absolute values for control (no swallow-wort tissue present) are shown in the left-hand column, followed by values for treatments exposed to swallow-wort tissues. Each value is the mean from two repetitions and three replicates (Petri dishes) per repetition. Significance of difference from control is indicated by *, **, *** for P < 0.1, 0.05, and 0.001, respectively. Where 0.1 < P < 0.05 the actual value of significance is given; coefficient of variation (CV) for root data = 82.32, for shoot data = 70.26.

Root Length (% of control)							
Indicator Species	Control (mm)	Root tissues		Stem tissues		Leaf tissues	
		BSW	*PSW*	*BSW*	*PSW*	*BSW*	*PSW*
Annual bluegrass	5.0	108.2	49.1	47.5	65.1	73.9	12.7
Barnyardgrass	19.9	134.5	60.5	123.0	60.9	79.8	71.4
Black swallow-wort	22.9	135.2 * (0.08)	144.7	83.0	212.0	61.3	49.7
Butterfly milkweed	10.0	127.2	52.1	24.4	71.5	18.0	17.4
Common milkweed	18.8	62.9	31.0	50.6	54.1	38.4	13.8 **
Large crabgrass	16.4	40.3 * (0.06)	53.9	56.8	60.0	58.7	32.4 **
Lettuce	17.8	83.8	49.1	110.0	63.2	46.5	40.8
Orchardgrass	27.6	68.3	26.9	56.5	94.0	13.9	15.8
Pale swallow-wort	9.2	96.3	90.0	54.3	50.7	4.7	10.6
Tomato	31.9	68.4	72.7	94.6	46.4	15.5 **	41.3
Shoot Length (% of control)							
Annual bluegrass	3.8	309.0	148.1	247.0	119.5 * (0.06)	164.7	59.1
Barnyardgrass	18.8	129.0	99.1	141.0	89.7	109.3	101.4
Black swallow-wort	11.5	113.0	282.3	91.7	319.2	132.2	276.4
Butterfly milkweed	11.3	164.3	111.3	57.0	153.9	62.8	82.4
Common milkweed	17.7	111.0	87.8	93.7	92.0	114.9	58.1
Large crabgrass	20.5	61.6	84.3	96.7	100.3	85.8	72.2
Lettuce	16.8	74.6	96.1	79.9	88.0	74.7	98.8
Orchardgrass	16.1	110.1	83.3	78.1	87.1	54.8	82.6
Pale swallow-wort	8.5	96.1	48.1	100.7	22.3	79.4	26.8
Tomato	24.0	90.0	91.1	113.1	62.2	59.5	72.2

Table A2a. Effect of 0.5 g of dried tissues (root, stem and leaf) collected from field stands of PSW and BSW plants on final germination and germination rate of indicator species. Indicator seeds were grown for 7 days in modified Parker bio-assays conducted under laboratory conditions. Absolute values for control (no swallow-wort tissue present) are shown in the left-hand column, followed by values for treatments exposed to swallow-wort tissues. Each value is the mean from two repetitions and three replicates (Petri dishes) per repetition. Significance of difference from control is indicated by *, **, *** for P < 0.1, 0.05, and 0.001, respectively. Where 0.1 < P < 0.05 the actual value of significance is given; coefficient of variation (CV) for final germination data = 36.2, for germination rate data = 47.36.

Indicator Species	Control (% of seeds)	Final Germination (% of control)					
		Root tissues		Stem tissues		Leaf tissues	
		BSW	*PSW*	*BSW*	*PSW*	*BSW*	*PSW*
Annual bluegrass	25.0	50.0	50.0	40.0	120.0	110.0	60.0
Barnyardgrass	44.4	82.1	77.8	100.0	110.8	136.8	93.1
Black swallow-wort	81.3	100.0	130.0 * (0.09)	62.5 **	120.0	100.0	130.0 * (0.09)
Butterfly milkweed	84.4	100.0	97.6	100.0	85.7	92.3	69.1
Common milkweed	95.0	100.0	95.0	94.4	100.0	82.4	95.0
Large crabgrass	85.6	83.0 * (0.09)	101.3	84.9	105.1	92.5	105.3
Lettuce	95.6	96.4	96.7	98.2	90.0	92.9	94.8
Orchardgrass	35.0	82.4	163.6	117.6	145.5	129.4	136.4
Pale swallow-wort	83.8	100.0	92.9	87.5	92.9	102.1	85.7
Tomato	97.8	98.3	91.4	101.7	91.6	87.9	94.9
Control (# seeds/day)		Germination Rate (% of control)					
Annual bluegrass	1.5	50.8	43.9	35.3 * (0.05)	123.9	97.8	65.5
Barnyardgrass	2.3	90.7	77.5	105.3	120.0	139.0	96.1
Black swallow-wort	1.9	98.6	140.2 * (0.06)	65.3	136.6	85.7	136.1
Butterfly milkweed	1.5	95.3	116.5	102.2	92.7	67.4	90.6
Common milkweed	1.4	97.9	187.7 **	105.6	99.5	56.1	209.7 **
Large crabgrass	5.1	76.4 **	103.6	83.2	103.3	91.1	102.6
Lettuce	3.7	113.6	78.7	93.5	85.4	107.6	63.7
Orchardgrass	2.2	81.7	177.0	107.4	150.9	119.8	120.4
Pale swallow-wort	2.1	94.2	95.6	96.5	101.1	112.4	95.1
Tomato	6.0	95.2	92.1	98.9	93.8	85.9	89.7

Table A2b. Effect of 1 g of dried tissues (root, stem and leaf) collected from field stands of PSW and BSW plants on the final germination and germination rate of indicator species. Indicator seeds were grown for 7 days in modified Parker bio-assays conducted under laboratory conditions. Absolute values for control (no swallow-wort tissue present) are shown in the left-hand column, followed by values for treatments exposed to swallow-wort tissues. Each value is the mean from two repetitions and three replicates (Petri dishes) per repetition. Significance of difference from control is indicated by *, **, *** for P < 0.1, 0.05, and 0.001, respectively. Where 0.1 < P < 0.05 the actual value of significance is given; coefficient of variation (CV) for final germination data = 29.81, for germination rate data = 41.58.

Indicator Species	Control (% of seeds)	Final Germination (% control)					
		Root tissues		Stem tissues		Leaf tissues	
		BSW	*PSW*	*BSW*	*PSW*	*BSW*	*PSW*
Annual bluegrass	25.0	60.0 * (0.07)	53.3 * (0.05)	60.0	60.0 * (0.07)	66.7	80.0
Barnyardgrass	44.4	94.7	85.9	63.2 * (0.08)	84.2	107.4	67.3
Black swallow-wort	81.3	93.8	150.0 **	93.8	130.0	87.5	120.0
Butterfly milkweed	84.4	69.2	63.5	107.7	66.7	46.2 **	69.1
Common milkweed	95.0	88.9	95.0	80.6 **	95.0	100.0	90.0
Large crabgrass	85.6	86.8	96.8	94.3	106.6	98.1	101.5
Lettuce	95.6	96.4	85.7	85.7	100.1	101.8	94.8
Orchardgrass	35.0	70.6	72.7	141.2	127.3	70.6	121.2
Pale swallow-wort	83.8	81.3	71.4	62.5	50.0 **	52.1 * (0.07)	21.4
Tomato	97.8	101.7	94.9	94.8	96.6	96.6	95.0
Control (# seeds/day)		Germination Rate (% control)					
Annual bluegrass	1.5	53.0 **	57.2 * (0.06)	62.7	65.5	51.83 **	72.7
Barnyardgrass	2.3	97.1	94.9	71.3	85.6	115.7	68.7
Black swallow-wort	1.9	97.3	163.7 **	96.1	148.8 **	98.0	130.3
Butterfly milkweed	1.5	74.5	82.8	113.4	87.6	50.8	102.0
Common milkweed	1.4	99.4	200.1 **	97.1	164.0 * (0.09)	95.6	175.0 **
Large crabgrass	5.1	85.8	93.3	87.9	107.0	89.6	97.5
Lettuce	3.7	125.0	59.6	92.0	94.7	136.0	84.3
Orchardgrass	2.2	65.1	71.4	138.0	130.5	51.0	122.1
Pale swallow-wort	2.1	80.1	73.6	60.8	54.1 **	43.2 **	80.5
Tomato	6.0	98.4	94.1	92.4	95.7	95.1	89.1 **

REFERENCES

Altieri, M (1995) Agroecology: the science of sustainable agriculture, 2nd Ed. Boulder, CO: Westview Press. 433 pp

Bais, HP, R Vepachedu, S Gilroy, RM Callaway, JM Vivanco (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science 301: 1377-1380

Bertin, C, RN Paul, SO Duke, LA Weston (2003a) Laboratory assessment of the allelopathic effects of fine leaf fescues. Journal of Chemical Ecology 29: 1919-1937

Bertin, C, X Yang, LA Weston (2003b) The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256: 67-83

Blum, U (1995) The value of model plant-microbe-soil systems for understanding processes associated with allelopathic interaction. Inderjit, KMM Dakshini, CL Foy, Eds. Allelopathy: Organisms, Processes and Applications. Washington DC: American Chemical Society. Pp 127 – 131

Callaway, RM, ET Aschehoug (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521-523

Capo, M, JM Saa (1989) (-)-antofine: a phenanthroindolizidine from *Vincetoxicum nigrum*. Journal of Natural Products 52: 389-390

Cappuccino, N (2004) Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos 106: 3-8

Clinch, JJ, H.J Keselman (1982) Parametric alternatives to the analysis of variance. Journal of Educational Statistics 7: 207-214

D'Antonio, C, LA Meyerson (2002) Exotic plant species as problems and solutions in ecological restoration: a synthesis. Restoration Ecology 10: 703-713

DiTommaso A, Losey JE (2003) Oviposition preference and larval performance of

monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. Entomologia Experimentalis et Applicata 108: 205-209

DiTommaso, A, DC Brainard, BR Webster (2005a) Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration. Canadian Journal of Botany 83: 102-110

DiTommaso, A, FM Lawlor, SJ Darbyshire (2005b) The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [= *Vincetoxicum nigrum* (L.) Moench]. Canadian Journal of Plant Science 85: 243-263

Facelli, JM, STA Pickett (1991) Plant litter: its dynamics and effects on plant community structure. Botanical Review 57: 1-32

Fitter, AH, RKM Hay (1987) Environmental Physiology of Plants, 2nd Ed. San Diego, CA: Academic Press, Inc. 423 pp

Gordon, DR (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. Ecological Application 8: 975-989

Greipsson, S, A DiTommaso (2006) Invasive non-native plants alter the occurrence of arbuscular mycorrhizal fungi and benefit from this association. Ecological Restoration 24: 236-241

Hodge, A, P Millard (1998) Effect of elevated CO_2 on carbon partitioning and exudates release from *Plantago lanceolata* seedlings. Physiologia Plantarum 103: 280-286

Inderjit, J Weiner (2001) Plant allelochemical interference or soil chemical ecology? Perspectives in Plant Ecology, Evolution and Systematics 4: 3-12

Inderjit, LA Weston (2003) 10. Root exudates: an overview. de Kroon, H, EJW Visser Eds. Root Ecology – Ecological Studies, Vol. 168. Berlin-Heidelberg,

Germany: Springer-Verlag. Pp. 235 - 255

Inderjit, RM Callaway, JM Vivanco (2006) Can plant biochemistry contribute to understanding of invasion ecology? Trends in Plant Science 11(: 574-580

Kruse, M, M Strandberg, B Strandberg (2000) Ecological effects of allelopathic plants: a review. National Environmental Research Institute (NERI) Technical Report No. 315. Silkeborg, Denmark: NERI. 66 pp

Langheim, JH (1994) Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of Chemical Ecology 20: 1223-1279

Lawlor, FM (2000) Herbicidal treatment of the invasive plant *Cynanchum rossicum* and experimental post control restoration of infested sites. MSc Thesis, State University of New York, College of Environmental Science and Forestry, Syracuse, NY. 77 pp

Lee, SK, KA Nam, YH Heo (2003) Cytotoxic activity and G2/M cell cycle arrest mediated by antofine, a phenanthroindolizidine alkaloid isolated from *Cynanchum paniculatum*. Planta Medica 69: 21-25

Lumer, C, SE Yost (1995) The reproductive biology of *Vinctoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York State. Bulletin of the Torrey Botanical Club 122: 15-23

Mattila HR, Otis GW (2003) A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog-strangling vine (*Vincetoxicum rossicum*). Entomologia Experimentalis et Applicata 107: 193-199

McKague CI, N Cappuccino (2005) Response of pale swallow-wort, *Vincetoxicum rossicum*, following aboveground tissue loss: implications for the timing of mechanical control. Canadian Field Naturalist 119: 525-531.

Mitchell CE, AA Agrawal, JD Bever, GS Gilbert, RA Hufbaeur, JN Klironomos, JL

Maron, WF Morris, IM Parker, AG Power, EW Seabloom, ME Torchin, Dp Vasquez (2006) Biotic interaction and plant invasions. Ecology Letters 9: 726-740

Newman, EI, AD Rovira (1975) Allelopathy among some British grassland species. Journal of Ecology 63: 727-738

Nilsson, MC (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum* Hagerup. Oecologia 98: 1-7

Nowak, R, W Kisiel (2000) Hancokinol from *Vincetoxicum officinale*. Fitoterapia 71: 584-586

Orr, SP, JA Rudgers, K Clay (2005) Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecology 181: 153-165

Parker, C (1966) The importance of shoot entry in the action of herbicides applied to the soil. Weeds 14: 117-121

Putnam, AR, LA Weston (1986) Adverse impacts of allelopathy in agricultural systems. Putnam, AR, CS Tang, Eds. The Science of Allelopathy. New York, NY: John Wiley & Sons, Inc. Pp. 43-56

Radosevich, S, J Holt, C Ghersa (1997) Weed Ecology: Implications for Management, 2nd ed. New York, NY: John Wiley & Sons, Inc. 589 pp

Renne, IJ, BG Rios, JS Fehmi, BF Tracy (2004) Low allelopathic potential of an invasive forage grass on native grassland plants: a cause for encouragement. Basic and Applied Ecology 5: 261-269

Rice, EL (1984) Allelopathy, 2nd ed. Orlando, FL: Academic Press, Inc. 422 pp

Rice, EL (1986) Allelopathic growth stimulation. Putnam, AR, CS Tang, Eds. The Science of Allelopathy. New York, NY: John Wiley & Sons, Inc. Pp. 23-42

Roberts, KJ, RC Anderson (2001) Effect of garlic mustard [*Alliaria petiolata* (Beib.

Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146: 146-152

Schulz, M, A Friebe (1999) Detoxification of allelochemicals in higher plants and enzymes involved. Inderjit, KMM Dakshini, CL Foy, Eds. Principles and Practices in Plant Ecology: Allelochemical Interactions. Boca Raton, FL: CRC Press LLC. Pp 383 - 400

Sheeley SE (1992) Life history and distribution of *Vincetoxicum rossicum* (Asclepiadaceae): An exotic plant in North America. MSc Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY 126 pp

Smith, LL (2006) Arbuscular mycorrhizal fungi and the competitiveness of the invasive vine PSW (*Vincetoxicum rossicum*). MSc Thesis, Cornell University, Ithaca, NY. 85 pp

Smith, LL, A DiTommaso, J Lehmann, S Greipsson (2008) Effects of arbuscular mycorrhizal fungi on the exotic invasive vine pale swallow-wort (*Vincetoxicum rossicum*). Invasive Plant Science and Management 1(2) (In press)

Sonneveld, C, N Straver (1994) Nutrient solutions for vegetables and flowers grown in water or substrates. Vol 8. Naaldwijk, The Netherlands. 45 pp

Staerk, D, J Christensen, E Lemmich, JO Duus, CE Olsen, JW Jaroszewski (2000) Cytotoxic activity of some phenanthroindolizidine *N*-oxide alkaloids from *Cynanchum vincetoxicum*. Journal of Natural Products 63: 1584-1586

Staerk, D, AK Lykkeberg, J Christensen, BA Budnik, F Abe, JW Jaroszewski (2002) In vitro cytotoxic activity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against drug-sensitive and multidrug-resistant cancer cells. Journal of Natural Products 65: 1299-1302

Stevens, KL, GB Merrill (1985) Sesquiterpene lactones and allelochemicals from *Centaurea*. Thompson, AC, Ed. The Chemistry of Allelopathy. Washington, DC: American Chemical Society. Pp. 83-98

Stinson, KA, SA Campbell, JR Powell, BE Wolfe, RM Callaway, GC Thelen, SG Hallett, D Prati, JN Klironomos (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PloS Biology 4(5): e140. DOI: 10.1371/journal.pbio.0040140

Stowe, LG (1979) Allelopathy and its influence on the distribution of plants in an Illinois old-field. Journal of Ecology 67: 1065-1085

Van Andel, J, J Aronson, Eds (2005) Restoration Ecology. Oxford, UK: Blackwell Publishing. 336 pp

Wardle, DA, KS Nicholson, A Rahman (1996) Use of a comparative approach to identifying allelopathic potential and relationship between allelopathy bioassays and "competition" experiments for ten grassland and plant species. Journal of Chemical Ecology 22: 933-948

Wardle, DA, MC Nilsson, C Gallet, O Zakrisson (1998) An ecosystem-level perspective of allelopathy. Biological Reviews 73: 305-319

Weidenhamer, JD (1996) Distinguishing resource competition and chemical interference: overcoming the methodological impasse. Agronomy Journal 88: 866-875

Welch, BL (1938) The significance of the difference between two means when the population variances are unequal. Biometrika 29: 350-362

Welch, BL (1947) The generalization of Student's problem when several different population variances are involved. Biometrika 34: 29-35

Weston, LA, AR Putnam (1986) Inhibition of legume seedling growth by residues and extracts of quackgrass (*Agropyron repens*). Weed Science 34: 366-372

Weston, LA, R Harmon, S Mueller (1989) Allelopathy potential of sorghum-sudangrass hybrid (Sudex). Journal of Chemical Ecology 15: 1855-1865

Weston, LA, JN Barney, A DiTommaso (2005) A review of the biology, ecology and potential management of three important invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant and Soil 277: 53-69

Wink, M, B Latz-Bruning (1995) Allelopathic properties of alkaloids and other natural products: possible modes of action. Inderjit, KMM Dakshini, CL Foy, Eds. Allelopathy: Organisms, Processes and Applications. Washington DC: American Chemical Society. Pp 117 – 126

Wink, M, B Latz-Bruning, T Schmeller (1999) Biochemical effects of allelopathic alkaloids. Inderjit, KMM Dakshini, CL Foy, Eds. Principles and Practices in Plant Ecology: Allelochemical Interactions. Boca Raton, FL: CRC Press LLC. Pp 411 - 422

Wolfe, BE, JN Klironomos (2005) Breaking new ground: soil communities and exotic plant invasions. BioScience 55: 477-487

Zimmerman, DW, BD Zumbo (1993) Rank transformations and the power of the Student t test and the Welch t' test for non-normal populations with unequal variances. Canadian Journal of Experimental Psychology 47: 523-539

Genetic and Morphological Diversity in Relation to Environment for Populations of the Invasive Species Pale swallow-wort (*Vincetoxicum rossicum*) and Black swallow-wort (*V. nigrum*)

Pale and black swallow-wort are introduced perennial vines invasive throughout much of the northeastern US and southern Canada. While at the time of their introduction distributions of the two species were largely distinct, dispersal has resulted in ranges that are beginning to overlap. Although little is known about the species' genetics, adaptive morphological plasticity in response to light and other environmental factors is well documented for both species. Our objective was to characterize genetic diversity levels in introduced populations of the species, and also to identify whether these genotypic patterns or the environment (i.e. soil and climate) explained morphological variations of swallow-wort populations in New York. We found that the two species are monophyletic, and that intra-specific genetic similarity is high (mean = 0.79; Nei & Li's coefficient of genetic similarity). Genetic diversity did not explain morphological patterns in swallow-wort populations. Rather, adaptive morphological traits of invasive swallow-worts were negatively correlated with soil pH (plant height: $\beta_1 = -0.67$, $r^2 = 0.5$, $P < 0.05$) and positively correlated with precipitation levels (plant height: $\beta_1 = 0.51$, $r^2 = 0.52$, $P < 0.0037$; leaf shape: $\beta_1 = 0.55$, $r^2 = 0.52$, $P < 0.05$). Also, in comparison to sites invaded by BSW, sites with PSW infestations were characterized by significantly ($P < 0.05$) higher soil pH (13%), Ca (475%), and Al (369%) levels.

INTRODUCTION

The success of many invasive species can be attributed to phenotypic plasticity (Baker 1974; Daehler 2003; Maron et al. 2004; Richardson and Pysek 2006). In

particular, several authors have proposed that adaptive morphological traits that could facilitate invasion across multiple habitats - especially those that are resource limited - would be advantageous for invasive species (Burns and Winn 2006; Chun et al. 2007). However, adaptive evolutionary changes that enhance (or 'create') an invasive plant species, such as intraspecific hybridization, have also been well documented (Bossdorf et al. 2005; Ellstrand and Schierenbeck 2000; Gammon et al. 2007; Hollingsworth et al. 1999; Lee 2002).

Vincetoxicum rossicum (Kleopow) Babar (pale swallow-wort (PSW)) and *V. nigrum* (L.) Moench (black swallow-wort (BSW)) were introduced to North America in the late nineteenth century from distinct regions in central and southwestern Europe, respectively (DiTommaso et al. 2005b; Sheeley 1992). While their original history of introduction is unknown, it is believed that the species were brought as botanical specimens for gardens, from which they subsequently escaped (DiTommaso et al. 2005b; Lauvanger and Borgen 1998; Monanchino 1957; Sheeley and Raynal 1996). The species were probably introduced repeatedly during the late 1800s and early 1900s (Sheeley 1992; Smith 2006).

Morphologically the two species are almost identical, which has led to frequent disagreements as to their appropriate taxonomical placements (DiTommaso et al. 2005b; Lauvanger and Borgen 1998; Sheeley and Raynal 1996). PSW stems are typically 60-200 cm long, with opposite leaves that are 7-12 cm long and 5-7 cm wide (DiTommaso et al. 2005b). BSW stems are 4-200 cm long, and opposite leaves are normally 5-12 cm long and 2-6.5 cm wide. Flower buds in PSW are ovoid, the corona is distinctly lobed and petals are pink to maroon and hairless (DiTommaso et al. 2005b). BSW flower buds are more globose with an indistinctly lobed corona, and purple to black petals that are hairy along the inner surface (DiTommaso et al. 2005b; Lumer and Yost 1995). Reproduction in both species occurs primarily via follicle-

borne seeds with coma that facilitates wind dispersal (Cappuccino et al. 2002; DiTommaso et al. 2005a).

Vincetoxicum species are known to have relatively specialized insect pollinators associated with their complicated floral structures, although work in New York with BSW found that unspecialized fly species predominated as pollinators (Lumer and Yost 1995). The occurrence of insect pollination has led to the assumption that reproduction in swallow-worts (SWs) occurs primarily via out-crossing (Leimu 2004). However, in North America both species are reported to be self-fertile, and there is also evidence of vegetative reproduction, especially in black swallow-wort (Cappuccino 2004; DiTommaso et al. 2005b; Leimu 2004; Lumer and Yost 1995). PSW populations in Ottawa, Canada were reported to have a chromosome count of $2n = 22$ (Moore 1959). No chromosome counts have been reported for New World populations of BSW, though work in Spain and Italy has reported that the species is variably haploid ($n = 11$), diploid ($2n = 22$) or tetraploid ($2n = 44$) (Aparicio and Silvestre 1985; Diosdado et al. 1993; Moore 1959).

Both species have a wide tolerance to light and moisture conditions. It appears that SWs grow most aggressively in disturbed, open habitats and on drier, shallow soils where other plants do not grow well (Davis et al. 2000; DiTommaso et al. 2005b; Lawlor and Raynal 2002; Sheeley 1992; Smith 2006). Smith (2006) concluded that the ability of PSW to tolerate varying light and nutrient conditions was facilitated by its large root crown and ability to favorably alter soil mycorrhizal communities. Similarly, many invasive species allocate more resources to vegetative growth - often delaying reproduction - which increases overall fitness in later years (Chun et al. 2007). The two SW species exhibit considerable plasticity in morphology and other features in response to light including stem and internode lengths, leaf

shapes, overall reproductive output, and seed viability (Lawlor 2000; Sheeley 1992; Smith 2006).

Both species have invaded sites in distinct climatic areas of New York, from Long Island which is warmer and relatively dry to St. Lawrence County in northern NY which is typically cold and wet (New York State Climate Office 2008). While PSW is endemic to a region of the Ukraine typified by colder and wet weather, the Mediterranean BSW has shown a greater ability to survive and spread into areas in North America outside of its typical climatic range (DiTommaso et al. 2005b; World Meteorological Organization 2008).

Despite a growing body of literature on invasive *Vincetoxicum* species in North America, no one has yet investigated the genetic diversity of introduced populations, nor conducted morphology and habitat characterizations of sites outside central New York. We aimed to characterize the extent of variations found at locations invaded by the two *Vincetoxicum* species throughout their increasingly wide ranges in New York. The primary objective of this work was to identify which of these factors (genetics, plant morphology or environmental conditions) most influences patterns of SW invasion. The hypotheses tested were specifically:

(1) The two SW species are genetically distinct.

(2) Intra-species genetic diversity levels of SW populations will be high, with strong negative correlations between genetic similarity and distance between collection sites in particular.

(3) Variability in genetic diversity, rather than climate or soil characteristics, will best explain morphological variations of sampled SW populations.

MATERIALS AND METHODS

Soil and Plant Tissue Collection Protocols. PSW sites (14) and 9 BSW sites in New York were chosen based on reports of dense infestations at those locations by collaborators and landowners (**Figure 3.1**). Selected sites represented the full extent of the known ranges of both species within the state. At each site, a representative soil sample was collected using a 20 mm probe inserted to 15-20 cm depth. Samples (20-25) were taken at each site at points equidistant along transects that represented the entire stand of plants. The samples were pooled and homogenized using a trowel and the master sample was placed in a Ziploc bag. Master soil samples were sifted in the laboratory using a 5mm mesh sieve and any collected debris discarded. Samples were air-dried and then submitted to the Cornell Nutrient Analysis Laboratory (CNAL - Ithaca, NY) for analysis of micro- and macronutrients, pH and organic matter levels.

Ten plants of the particular species encountered at each site were selected to spatially represent the entire infestation site. Plant height and the number of nodes on each selected plant were recorded. Ten leaves were harvested from each plant beginning with the first pair of true leaves and continuing upwards for the following four nodes. Leaf length and width were measured and all leaves were then sealed in a Ziploc bag. Leaves were immediately placed into a cooler filled with dry-ice for transport. A representative voucher specimen containing reproductive structures for each sampling location was collected and filed at the LH Bailey Hortorium (Cornell University, Ithaca, NY). All plant leaf tissues were placed into –80 °C storage in the laboratory until DNA extraction occurred, which took place no longer than four weeks after initial collection

DNA Extraction and Analysis. Genomic DNA was extracted from leaf samples using the hexadecyltrimethylammonium bromide (CTAB) method modified from

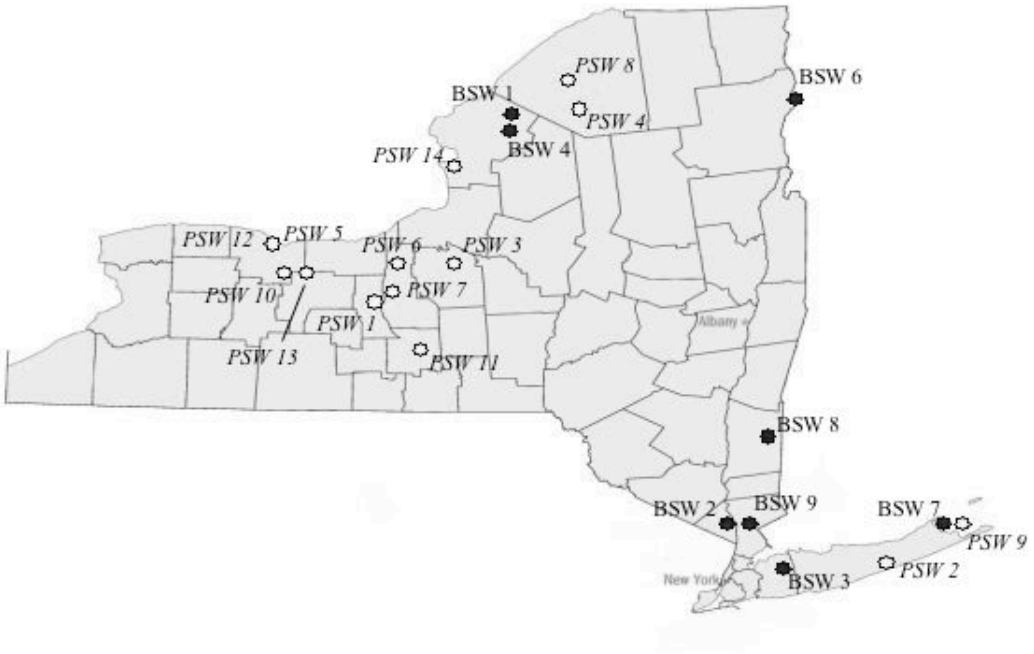


Figure 3.1. Swallow-wort sampling sites in New York, black stars represent black swallow-wort (BSW) sites and the white stars represent pale swallow-wort (PSW) sites. See **Appendix II, Table B1** for more detailed location information for each site.

Doyle (1991) and Bailey (2003). Initially, 10 µl β-mercaptoethanol was added to the 15 ml centrifuge tube containing the preheated (60-65 °C) CTAB buffer (5 ml). Frozen swallow-wort leaves were de-veined and ground in a ceramic mortar using liquid nitrogen; 1 g of finely powdered leaf tissue was added to the 15 ml centrifuge tube. Tubes were placed in a drying oven and incubated for 45 minutes at 60 °C.

Extraction with chloroform/iso-amyl alcohol (24:1) was repeated twice for each sample before precipitation of DNA using 2/3 volume of cold (-20 °C) isopropanol. The DNA pellet was removed from the centrifuge tube using a pipette tip and added to a 15 ml tube containing 5 ml CTAB wash buffer. Tubes were placed horizontally on a shaker and run at 35 RPM for 20 minutes. After removing the supernatant, the DNA pellet was allowed to air dry briefly and then re-suspended in 0.5 ml TE buffer. DNA was extracted again if the UV absorption ratio (A 260/280) was outside the range 1.7 - 2.

10 µl of extracted DNA from the ten samples at each site were pooled to create a master DNA sample for each sampled swallow-wort population. This master DNA sample was then used for subsequent primer selection and final genetic diversity analyses.

Primer Selection. Two sets of randomly amplified polymorphic DNA (RAPD) and sequence-related amplified polymorphic (SRAP) primers were initially screened for use, those designed to be 'universal' and those designed for specific invasive weedy species. Of this set of 63 primers, twelve decamer RAPD primers from McArthur et al. (1998) were chosen due to their high band quality, quantity and maximum reproducibility: UBC 157, UBC 180, UBC 285, UBC 302, UBC 356, UBC 358, UBC 361, UBC 421, UBC 571, UBC 584, UBC 595 and UBC 598. All primers were purchased from MWG Biotech Inc (High Point, NC).

DNA Amplification by PCR and Electrophoresis. Recombinant *Taq* DNA polymerase, polymerase chain reaction (PCR) buffer, and dNTP mixture were obtained from Takara Bio Inc (Madison, WI). PCR reaction mixture (24 μl) contained 2.4 μl 10X PCR buffer, 1.92 μl dNTP mix, 0.12 μl *Taq* polymerase, 13.16 μl de-ionized water, 4 μl template DNA and 2.4 μl of the appropriate primer. DNA amplification was performed in a Bio-Rad ICycler thermocycler (Bio-Rad Labs, Hercules, CA). The initial cycle was 3 mins at 92 °C; the subsequent 48 cycles consisted of 1 min at 86 °C, 105 sec at 36 °C and 2 mins at 72 °C. The final cycle was 7 min at 72 °C.

For electrophoretic separation of amplified DNA products 3.5 μl of bromophenol blue-based 10X DNA loading dye was added to the 24 μl PCR reaction tube containing the reaction mixture and vortexed. 18 μl of this mixture was loaded onto a 1.2% agarose gel containing 8 μl ethidium bromide submerged in TBE buffer, and run at 130 V for 75 mins. Amplification products were visualized on an Alpha Imager 2200 UV transilluminator (Alpha Innotech Corporation, San Leandro, CA). Amplification and separation of all samples was replicated twice and experimental runs were repeated over time.

DNA Analysis. Stained gels were visually scored for the presence (1) or absence (0) of co-migrating bands 0.2 to 2 kb in size. A standard DNA ladder (All-purpose Hi-Lo DNA marker 50-10,000 bp, Bionexus Inc, Oakland, CA) was used to measure the molecular weight of bands.

Pair-wise coefficients of genetic similarity were calculated using the following equation for Nei and Li's coefficient (Lamboy 1994 modified from Nei and Li 1979):

$$\text{NL coefficient} = \frac{2sp}{2sp + (1\text{-}s)} \quad (1)$$

where s is defined as the proportion of total bands that were shared by both sites in the pair-wise analysis, and p is the proportion of s that are actually present (scored as 1) for both sites.

Construction of phylogenetic trees was performed using PAUP 4.0b10 (Sinauer Associates, Inc Publishers, Sunderland, MA). Distance-based neighbor joining (NJ) method and optimality criteria-based maximum parsimony (MP) were used to create multiple trees in order to compare final results. For MP analysis random taxon addition (RTA) with 100 replicates was conducted. For both methods bootstrap analysis with 1,000 replicates was performed to find the optimal tree, and bootstrap values reported when greater than 50.

Climate and Geographic Data Collection. All climate data was obtained from publications of the National Oceanic and Atmospheric Administration's (NOAA) National Climate Data Center (NCDC). The NOAA-maintained weather station closest to each sampled swallow-wort site was chosen using lat long coordinates and the National Hurricane Center's (NHC) lat long calculator of the National Weather Service (http://www.nhc.noaa.gov/gccalc.shtml). Temperatures for the day that each site was sampled were from the Global Summary of the Day database (NOAA GSOD 2008) or monthly logs of climatological observations (NOAA Logs 2008) maintained for each NOAA weather station. 30 year averages for both temperature and precipitation were from the Monthly Station Norms 1971-2000 publication (NOAA NCDC 2000). Data for 2006 monthly temperature and precipitation were obtained from the "U.S. Climate at a Glance" database. Finally, freeze and frost data were from the "Freeze/Frost Data – CLIM20 supp no 1" report (NOAA NCDC 2008).

Pair-wise geographic distances for correlation analysis were calculated from the NHC latitude/longitude calculator.

Statistical Analysis. Simple t- tests or Tukey HSD tests were used for treatment means comparisons in some experiments. Pair-wise correlations were tested using the multi-variate analysis platform in JMP. Pair-wise correlation significances were tested using probability (P) values generated by Pearson product-moment correlations in the same analysis platform.

Because of correlations among plant morphology variables (means calculated for every variable at each site), soil analysis variables and climatic variables, principle components analysis (PCA) was used to reduce the dimensions of the three data sets. All variables were first standardized prior to performing PCA (Gotelli and Ellison 2004). The number of components extracted in each analysis was determined using the eigenvalue-one criterion and scree tests (Lehman et al. 2005). Extracted components were rotated using Varimax rotation and saved for regression analysis (Joliffe 2002). Rotated components of morphology variables were then regressed on the soil variables and climatic variables (Ellison et al. 2004). Significance of correlations was then tested using Pearson product-moment correlation P values.

All statistical analyses were conducted using JMP software (ver. 7 (2007), SAS Institute Inc., Cary, NC).

RESULTS

Swallow-wort Plant Morphologies. For both swallow-wort species, the largest leaves were found at sites with partial shade (**Table 3.1**). Under a full forest canopy, leaf width decreased 2.5% and length 3%. In full sun, leaf widths diminished 14% and leaves were 18% shorter. Similarly, mean internode lengths were significantly (P

Table 3.1. Summary of morphological data for black and pale swallow-wort plants from New York populations. Cover type is an approximation of the light availability at each site, with FSu: Full Sun, P: Partial sun, and FSh: Full Shade. Individual leaf width and length measurements are means from 100 leaves sampled at each site, with ten leaves per plant (10 plants per site) sampled. Leaf pair measures are the average for each pair of leaves at each node, with five nodes per sampled plant; β_1 is a linear regression of the average leaf pair shape index (SI) over the five nodes sampled vertically from the first pair of true leaves upwards. Means not connected by the same letter are significantly different at $P < 0.05$.

Site ID	Date Sampled	Cover Type	Mean Indiv Leaf Width (cm)	Mean Indiv Leaf Length (cm)	Mean Leaf Pair SI (W/L)	Mean Plant Height (cm)	Mean # of Nodes	Mean Internode Length (cm)
PSW 1	Jul 25	FSu	4.5 fghi	8.8 ghi	0.50 cdefg	105.2 bcde	12.7 bc	8.13 cdef
PSW 2	Aug 8	P	5.1 bcde	10.8 b	0.47 fg	107.2 bcde	13 bc	8.18 cdef
PSW 3	Jul 11	FSh	5.4 a	10.3 bcd	0.53 bcdef	110 abcde	11.5 c	9.54 abcd
PSW 4	Jun 22	P	5.1 bcd	10.2 bcd	0.50 defg	118.7 abcde	11.1 c	10.87 ab
PSW 5	Jul 25	P	4.5 efghi	8.9 gh	0.50 cdefg	99.3 cde	12.1 bc	8.45 bcdef
PSW 6	Jul 11	P	4.9 bcdefg	9.9 cde	0.49 defg	113.1 abcde	11.3 c	9.86 abcd
PSW 7	Jul 11	FSh	4.2 hi	8.7 ghi	0.48 efg	82.2 de	10.5 c	8.02 cdef
PSW 8	Jun 22	FSh	5.3 bc	10.2 2 cd	0.52 cdef	125.7 abcd	11.7 c	10.93 a
PSW 9	Aug 9	P	5.5 ab	12.5 5 a	0.43 g	157.2 ab	18.1 ab	8.95 abcde
PSW 10	Jul 10	FSu	4.8 bcdefgh	9.6 def	0.49 defg	103.1 bcde	11.3 c	9.23 abcde
PSW 11	Jun 29	P	5.9 a	10.5 bc	0.56 bcd	143.3 abc	13.1 bc	11.05 ab
PSW 12	Jul 25	P	4.9 bcdefg	10.1 cd	0.47 fg	114.8 abcde	12.2 bc	9.39 abcd
PSW 13	Jul 10	FSu	4.4 fghi	8.6 ghij	0.51 cdefg	115 abcde	11.4 c	10.01 abc
PSW 14.1	Jun 26	P	5 bcdef	9.2 efg	0.54 bcde	100.1 cde	11.8 c	8.42 bcdef
PSW 14.2	Jun 26	FSu	4.7 cdefgh	8.2 ijk	0.57 bc	98.6 cde	13.1 bc	7.56 cdef
BSW 1	Jul 31	P	5.9 a	9.2 fg	0.64 a	Data unavailable		
BSW 2	Jul 17	FSu	4.1 hi	6.9 l	0.60 ab	102.5 bcde	13.8 bc	7.58 cdef
BSW 3	Aug 8	FSu	4 i	8.3 hijk	0.48 efg	86.6 de	14.2 bc	6.15 f
BSW 6	Jul 13	FSu	4.2 ghi	8 jk	0.53 bcdef	69.9 e	10.2 c	6.7 def
BSW 7	Aug 9	P	4.6 defgh	10.4 bc	0.44 g	137.5 abcd	20.7 a	6.61 ef
BSW 8	Jul 18	FSu	4.38 fghi	7.83 k	0.55 ± 0.13 bcd	164.3 a	20.2 a	8.02 cdef
BSW 9	Jul 17	FSu	4.63 defgh	7.79 k	0.58 ± 0.12 ab	153.9 abc	19.4 a	7.83 cdef

< 0.05) greater in partially and fully shaded locations than in full sun. Internodes of plants at fully shaded sites were 30% longer than in full sun. Plant heights did not differ significantly between the three cover types, though those in partially shaded locations were 8% taller than either full sun or fully shaded sites.

PSW leaves were 17% longer ($P < 0.05$) than BSW leaves, but mean widths did not vary between the species (**Table 3.2**). BSW plants were slightly taller than PSW (6%, NS), but had 23% ($P < 0.05$) shorter internodes. However, this finding may be due to a greater number (5/7) of the BSW sites sampled being in open fields.

Site Soil Characteristics. Of the eleven soil parameters analyzed (**Table 3.3**) only three differed significantly ($P < 0.05$) between the two SW species (**Table 3.2**). PSW sites had much higher (375%) soil calcium levels than did BSW invaded sites. Similarly, PSW sites had higher (269%) aluminum and pH (13%) levels. Analysis of individual pair-wise correlations between plant morphological and soil characteristics revealed that leaf widths were positively affected by higher nitrate levels (**Table 3.4**; $r^2 = 0.51$, $P < 0.025$). Plant height and the number of nodes per plant were negatively correlated with pH levels ($r^2 = - 0.49$, $P < 0.05$ and $r^2 = - 0.74$, $P < 0.0001$, respectively). Iron ($r^2 = 0.63$, $P < 0.025$), aluminum ($r^2 = 0.74$, $P < 0.0001$) and magnesium ($r^2 = - 0.51$, $P < 0.025$) were also significantly correlated with the number of nodes per SW plant.

Climate. Climate affected the two SW species similarly (**Table 3.5**). The coldest site sampled during the three month period (June – August) was PSW 4 in northern NY (St. Lawrence County, 15.8 °C on June 22). The warmest was BSW 3 on the eastern end of Long Island, 26.4 °C at the beginning of August. However, 2006 was slightly warmer and much wetter than either of the historical data-sets (**Appendix II, Figures**

Table 3.2. Summary of mean morphological data and soil chemical characteristics for 8 BSW sites and 14 PSW sites sampled in New York in the summer of 2006. Individual leaf width and length measurements for each site were the means of ten leaves per plant, with ten plants per site sampled. Leaf pair shape index (SI) values were the mean of five leaf pairs sampled per plant. Soil data are based on analysis of a single composite sample from each site, which in turn was an aggregate of 20-25 composite samples.

	BSW	PSW		BSW	PSW
Mean Indiv Leaf Width (cm)	4.55	4.93	P (mg/kg)	1.63	8.83
Mean Indiv Leaf Length (cm)	**8.34 b**	**9.77 a**	K (mg/kg)	57.17	76.53
Mean Leaf Pair SI	0.55	0.5	Mg (mg/kg)	127.63	168.85
Plant Height (cm)	119.10	112.90	Ca (mg/kg)	**862.17 b**	**4098.73 a**
# of Nodes	**16.42 a**	**12.33 b**	Fe (mg/kg)	2.98	4.58
Internode Length (cm)	**7.19 b**	**9.34 a**	Al (mg/kg)	**22.77 b**	**83.98 a**
			Mn (mg/kg)	14.93	23.92
			Zn (mg/kg)	2.19	3.07
			pH	**6.03 b**	**6.81 a**
			% OM	3.74	5.24
			NO3 (mg/kg)	9.97	15.54

Table 3.3. Summary of soil chemical characteristics for 15 PSW sites and 6 BSW sites sampled across New York in 2006.

Site ID	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Fe (mg/kg)	Al (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	pH	% OM	NO3 (mg/kg)
PSW1	26.3	181	262.7	12655	1.5	8.7	36.7	2.9	7.4	6.3	35.6
PSW2	2.1	40	76.2	663	5.4	43	10.4	3.6	5.2	2.5	12.9
PSW3	1.3	55	116.3	4391	7.3	67.3	26.9	2	6.3	9.3	12.3
PSW4	4.3	42	119	2053	1.5	19.4	19.7	5.6	7	3.3	15.5
PSW5	15.9	80	118.9	2176	1.8	9.9	13.1	2.2	7.2	2.1	15.3
PSW6	7.8	42	129.3	5667	1	8.8	20.8	0.8	7.6	2.2	12.2
PSW7	2.3	73	214.7	1667	2.6	11.2	17.2	0.3	7	2.8	6.4
PSW8	3.3	84	316.2	2127	2.3	7.1	13.6	2.2	7.2	4.1	30.7
PSW9	8	31	42.1	707	6.1	51.4	23.2	1.6	5.5	2.2	15.3
PSW10	2.2	37	161.3	2422	3.3	20.7	16.8	0.6	7.2	2.1	7
PSW11	24.7	117	214.3	7417	2.5	7.5	47	2.7	7.3	5.7	48.3
PSW12	20.1	149	307.6	4904	2.1	9.1	31.9	6	7.3	8.4	0
PSW13	2.4	67	120.5	654	3	35.7	20.7	0.7	6	2.1	5.4
PSW14.1	10.8	83	247.3	9918	1.7	13.7	38.1	1.5	7.1	17.8	10.2
PSW14.2	1	67	86.3	4060	2.6	28	22.7	0.3	6.7	7.7	6.1
BSW1	1.4	62	148.4	1672	3.1	44.1	7.6	2.4	6.8	3.5	24.8
BSW2	1	74	232.7	1097	0.7	13.3	9.9	0.8	7.2	2.8	0
BSW3	0.7	51	157.3	979	5.2	115.9	5	10.9	6	4.7	10.2
BSW7	0.6	41	48.3	291	8.3	97.4	19.4	1.5	5.2	2.8	6.7
BSW8	2.6	65	106.5	522	4.2	83.8	34.5	1.6	5.4	4.5	18.1
BSW9	3.5	50	72.6	612	6	149.4	13.2	1.4	5.6	4.2	0

Table 3.4. Linear pair-wise correlations of swallow-wort population and morphological characteristics and soil chemical properties, including macro- and micronutrients, pH levels and organic matter content. Population characters include: site; SW species present at site; date sampled and cover type. Morphological measures include: individual leaf width and length; the shape index (width/length) of each leaf pair; plant height, and the number of nodes. Significant correlations are indicated by *, ** and *** at the given *P* values: * - 0.05; ** - 0.025; *** - 0.001.

	Site	SW Species	Date Sampled	Cover Type	Indiv Leaf Width	Indiv Leaf Length	Leaf Pair SI	Plant Height	# of Nodes
P	-0.32	-0.39	-0.22	-0.02	0.29	0.19	-0.03	0.07	-0.19
K	-0.19	-0.23	-0.33	0.04	-0.02	-0.16	0.16	-0.13	-0.25
Mg	-0.17	-0.24	**-0.48***	-0.21	-0.02	-0.16	0.18	-0.34	**-0.51****
Ca	-0.36	**-0.44***	-0.43	-0.03	0.24	0.04	0.15	-0.22	-0.38
Fe	0.28	0.38	**0.66****	-0.10	0.14	0.32	-0.30	0.39	**0.63****
Al	**0.61****	**0.74*****	**0.50****	0.30	-0.23	-0.18	0.05	0.41	**0.74*****
Mn	-0.04	-0.31	-0.40	-0.06	0.45	0.19	0.18	0.32	0.00
Zn	-0.02	0.18	0.30	0.07	-0.10	0.08	-0.28	-0.19	-0.06
pH	-0.41	**-0.5***	**-0.67*****	-0.16	0.06	-0.12	0.21	**-0.49***	**-0.74*****
% OM	0.08	-0.17	-0.38	-0.12	0.22	-0.05	0.31	-0.16	-0.17
NO3	-0.38	-0.31	-0.25	-0.20	**0.51****	0.32	0.03	0.24	-0.11

Table 3.5. Summary of temperature, precipitation and freeze free period data for 14 PSW and 8 BSW sites sampled in New York.

Site ID	Date Sampled (2006)	Nearest NOAA Weather Station No. (distance to, km)	Monthly Temp 30 yr Norm (C)	2006 Monthly Temp Avg. (C)	Actual Mean Temp Day Sampled (C)	Monthly Precip 30 yr Norm (cm)	2006 Monthly Precip Avg. (cm)	Frost Out (Spring) Day Norm (Date)	Frost In (Fall) Day Norm (Date)	Freeze Free Period Norm (Days)
PSW 1	Jul 25	110 (45)	Data unavailable	21.78	Data unavailable	9.88	12.9	Data unavailable	Data unavailable	Data unavailable
PSW 2	Aug 8	152 (34)	22.44	19.44	Data unavailable	11.43	10.77	Data unavailable	Data unavailable	Data unavailable
PSW 3	Jul 11	191 (9)	21.61	21.78	22.22	10.21	12.9	Apr 18	Oct 28	148
PSW 4	Jun 22	81 (8)	17.39	18.17	15.83	8.08	18.08	May 4	Oct 4	105
PSW 5	Jul 25	168 (22)	21.50	21.78	25.39	7.44	12.9	Apr 16	Oct 27	147
PSW 6	Jul 11	183 (49)	Data unavailable	21.78	Data unavailable	9.47	12.9	Data unavailable	Data unavailable	Data unavailable
PSW 7	Jul 11	11 (47)	21.78	21.78	22.5	8.41	12.9	Apr 22	Oct 31	146
PSW 8	Jun 22	36 (18)	17.72	18.17	20	8.33	18.08	May 4	Oct 6	103
PSW 9	Aug 9	86 (7)	22.39	19.44	Data unavailable	11.66	10.77	Mar 22	Nov 19	150
PSW 10	Jul 10	168 (13)	21.50	21.78	22.61	7.44	12.9	Apr 16	Oct 27	147
PSW 11	Jun 29	99 (25)	18.00	18.17	20	9.83	18.08	May 1	Oct 17	123
PSW 12	Jul 25	168 (24)	21.50	21.78	25.39	7.44	12.9	Apr 16	Oct 27	147
PSW 13	Jul 10	202 (17)	Data unavailable	21.78	Data unavailable	7.49	12.9	Data unavailable	Data unavailable	Data unavailable
PSW 14	Jun 26	210 (68)	18.50	18.17	20.39	8.64	18.08	Apr 23	Oct 18	139

Table 3.5 (Continued)

Site ID	Date Sampled (2006)	Nearest NOAA Weather Station No. (distance to, km)	Monthly Temp 30 yr Norm (C)	2006 Monthly Temp Avg. (C)	Actual Mean Temp Day Sampled (C)	Monthly Precip 30 yr Norm (cm)	2006 Monthly Precip Avg. (cm)	Frost Out (Spring) Day Norm (Date)	Frost In (Fall) Day Norm (Date)	Freeze Free Period Norm (Days)
BSW 1	Jul 13	81 (23)	19.94	21.78	18.89	8.05	12.9	May 4	Oct 4	105
BSW 2	Jul 17	227 (44)	22.39	21.78	25.83	11.76	12.9	Apr 8	Nov 4	162
BSW 3	Aug 8	125 (39)	22.67	19.44	26.39	9.5	10.77	Mar 25	Nov 28	187
BSW 4	Jul 13	81 (22)	19.94	21.78	18.89	8.05	12.9	May 4	Oct 4	105
BSW 6	Jun 21	155 (5)	18.50	18.17	17.5	8.48	18.08	Apr 29	Oct 11	124
BSW 7	Aug 9	86 (6)	22.39	19.44	Data unavailable	11.66	10.77	Mar 22	Nov 19	150
BSW 8	Jul 18	124 (43)	20.39	21.78	Data unavailable	11.1	12.9	Apr 20	Oct 16	118
BSW 9	Jul 17	227 (15)	22.39	21.78	25.83	11.76	12.9	Apr 8	Nov 4	162

B1 and B2). Rainfall at several sites throughout central and northern New York was ten centimeters (18.1 cm) above historical monthly averages, although sites on Long Island received slightly less rainfall than typical. Freeze free periods varied considerably between sites, with PSW 8 in northern NY only experiencing 103 days without frost, while sites on Long Island and in the Hudson Valley having almost two more months without frost (**Table 3.5**).

Plant height was positively correlated (**Table 3.6**; $r^2 = 0.38$, $P < 0.025$) with the historical (30 yr) precipitation average. The number of nodes per plant was also strongly positively correlated with the historical rainfall mean ($r^2 = 0.7$, $P < 0.0001$). Plants measured at sites with later final frost dates in the spring had slightly wider leaves ($r^2 = 0.38$, $P < 0.025$) and produced more nodes ($r^2 = 0.74$, $P < 0.025$) though overall height was not affected by last spring frost.

Effects of Variations in Soil and Climate on Morphology. Three principal components accounted for 98% of the variation in plant morphology variables by site (**Table 3.7**). The first component reflected differences among the sites in mean leaf widths and lengths. The second explained variation based on plant height and the number of nodes per plant, while the third described variations between the sites in leaf shape (SI). Variation in soil characteristics was summarized by four principal components, which accounted for 85% of the variation between the sampled sites. The first component explained almost half of the total variation (48%) and described differences in pH and magnesium levels between sites. Other macronutrients (P, K, Ca, Mn and NO_3) varied considerably by site as well, and were accounted for by the second principal component. The third component reflected variability between sites in soil organic matter content, while the final principal component explained differences based on zinc levels in the soil.

Table 3.6. Linear pair-wise correlations of swallow-wort population and morphological characteristics and various climate-related measurements. Population characters include: site; SW species present at site; date sampled and cover type. Morphological measures include: individual leaf width and length; the shape index (width/length) of each leaf pair; plant height and the number of nodes. Climate measures include temperature and precipitation means (both long-term and annual) and characteristics of frost and freeze periods for each site. Significant correlations are indicated by *, ** and *** at the given *P* values: * - 0.05; ** - 0.025; *** - 0.001.

	Site	SW Species	Date Sampled	Cover Type	Indiv Leaf Width	Indiv Leaf Length	Leaf Pair SI	Plant Height	# of Nodes
Monthly Temp (30 yr)	0.17	0.39	**0.79*****	0.19	-0.56	-0.43	-0.10	-0.12	0.40
2006 Monthly Temp	-0.11	0.08	0.47	-0.04	-0.32	-0.20	-0.12	0.03	0.23
Mean Temp Day Sampled	0.27	0.34	**0.69****	0.21	-0.44	-0.40	0.02	0.07	**0.55***
Monthly Precip (30 yr)	0.47	0.63	**0.16****	0.24	-0.06	-0.49	0.76	**0.38****	**0.70*****
2006 Monthly Precip	-0.04	-0.3	**-0.83*****	-0.11	0.50	0.28	0.27	0.11	-0.31
Frost Out Date	-0.20	-0.19	**-0.74*****	-0.27	0.55	0.29	**0.38****	0.62	0.05
Frost In Date	0.51	0.74	**0.51****	0.39	-0.44	-0.58	0.34	0.25	**0.74****
Freeze Free Period	0.42	0.51	**0.82****	0.43	**-0.60****	-0.55	0.03	-0.20	0.50

Table 3.7. Results of principal components analysis of morphological variables, soil chemical variables and climatic variables. For the 5 morphological variables, the first three principal components (PC) accounted for 98% of the variances in the data. For the 11 soil analysis variables, the first four principal components accounted for 85% of the variance in the data. Finally, for the 8 climatic variables, the first three principal components accounted for 94% of the variance in the data. Values shown are the loadings on each variable on each of the principal components. All components were rotated using Varimax rotation.

Variable	PC 1	PC 2	PC 3	PC 4
Swallow-wort plant morphology				
Indiv Leaf Width	**0.993**	0.079	-0.018	
Indiv Leaf Length	**0.770**	0.082	-0.630	
Leaf Pair SI	-0.068	0.024	**0.995**	
Plant Height	0.414	**0.879**	0.065	
# of Nodes	-0.130	**0.972**	-0.047	
Site soil analysis				
P	0.337	**0.775**	0.329	0.125
K	0.467	**0.570**	0.428	0.249
Mg	**0.749**	0.177	0.328	0.315
Ca	0.400	**0.516**	0.661	-0.030
Fe	-0.922	-0.102	-0.025	0.050
Al	-0.843	-0.247	-0.029	0.269
Mn	0.031	**0.619**	0.654	-0.245
Zn	-0.084	0.063	-0.008	**0.949**
pH	**0.919**	0.157	0.140	-0.024
% OM	0.074	-0.031	**0.948**	0.067
NO3	0.111	**0.876**	-0.087	0.023
Climate				
Monthly Temp (30 yr Norm)	**0.810**	0.494	0.304	
2006 Monthly Temp	**0.978**	-0.001	0.032	
Actual Mean Temp Day Sampled	0.553	**0.638**	0.401	
Monthly Precip (30 yr Norm)	0.001	0.071	**0.937**	
2006 Monthly Precip	-0.919	-0.280	-0.146	
Frost Out Date	-0.080	-0.979	0.111	
Frost In Date	0.288	0.125	**0.880**	
Freeze Free Period Norm	0.292	**0.857**	0.411	

Climate variability between sites was explained by three principal components that accounted for 94% of the variation. The first component described differences between sites based primarily on temperature (including both the historical norm and 2006 monthly average), while the second accounted for variability between sites in the temperature on the day the site was sampled and the freeze free period at the site. The last component explained between-site variability based on historical precipitation levels and the date of the first frost in the Fall.

Genetic Diversity. The 12 primers selected produced a total of 101 reproducible bands, with 75% of these being polymorphic (**Table 3.8**). The number of bands per primer ranged from 5 (UBC 157 and 421) to 13 (UBC 571). Of the total number of bands, 24% were found in every site for both species, indicating these traits were shared by both swallow-worts. On the other hand, 6% of bands were found in all PSW populations but no BSW populations, while 9% were found in BSW populations only. Another 12% of bands were found only in PSW populations, but were not shared by all sites and so could be used to find differences between these populations. Similarly, 9% of bands were found solely in BSW populations but were not found in all populations.

The SW populations sampled in this study for either species, are very similar to one another. Of the individual pair-wise genetic similarity coefficients, 82% were greater than 0.7 (**Figure 3.2**). The minimum value for NL coefficients by site of origin was 0.71 (PSW 13) and the maximum was 0.83 (PSW 1) (**Table 3.9**). Genetic similarity was negatively correlated with the mean geographic distance between populations (**Figure 3.3**; $r^2 = 0.08$, $P < 0.0001$), which varied considerably (**Table 3.10**; min = 208 km (PSW 7), max = 315 km (BSW 7)).

Table 3.8. List of RAPD bands characterized by molecular mass (bp) and their population frequency in parenthesis, where frequency is less than 17 the band is polymorphic.

Primer	Band (frequency)												
157	1050 (16)	950 (15)	800 (15)	650 (13)	251 (14)								
180	1950 (7)	1550 (16)	950 (16)	800 (1)	750 (17)	700 (10)	550 (17)	475 (17)	225 (7)				
285	1350 (14)	950 (15)	800 (15)	600(4)	400 (15)	275 (17)							
302	1600 (14)	750 (16)	700 (1)	650 (3)	600 (16)	550 (1)	475 (1)	450 (17)	300 (17)	225 (17)			
356	1550 (15)	1100 (15)	650 (7)	550 (8)	500 (8)	350 (15)							
358	1000 (17)	750 (10)	600 (7)	450 (17)	400 (17)	250 (17)							
361	1100 (7)	1000 (10)	900 (7)	800 (10)	750 (17)	550 (17)	450 (17)	400 (7)	250 (17)				
421	1100 (17)	900 (12)	750 (17)	500 (14)	350 (12)								
571	1350 (7)	1000 (8)	950 (9)	850 (9)	800 (1)	700 (15)	650 (1)	600 (14)	550 (17)	450 (12)	400 (2)	300 (10)	250 (17)
584	1300 (17)	1000 (6)	950 (1)	875 (10)	700 (17)	650 (4)	600 (1)	575 (8)	500 (2)				
595	1475 (2)	1200 (7)	1100 (2)	1000 (3)	925 (2)	850 (3)	800 (3)	700 (3)	675 (17)	625 (3)	550 (6)	475 (10)	
598	1550 (17)	900 (2)	800 (17)	750 (1)	625 (17)	525 (1)	500 (2)	475 (7)	400 (16)	350 (10)	250 (15)		

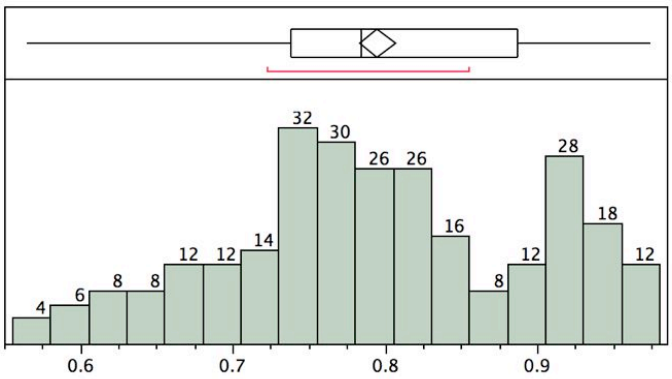


Figure 3.2. Distribution of Nei and Li's coefficients (NL) of genetic similarity for all swallow-wort populations on a scale of 0 (none) to 1 (identical). Coefficients were calculated from pair-wise analysis of RAPD primer band presence/absence of ten PSW populations and seven BSW populations in New York. Mean NL coefficient for all populations was 0.79 ± 0.1 (1 SD). Above each bar are counts of the number of pairs whose NL coefficient fell within the indicated range.

Table 3.9. Matrix of Nei and Li's (NL) coefficients of genetic similarity on a scale of 0 (none) to 1 (identical) for 10 pale swallow-wort populations and 7 black swallow-wort populations in New York.

	PSW 1	PSW 2	PSW 4	PSW 5	PSW 7	PSW 9	PSW 10	PSW 11	PSW 12	PSW 13	BSW 1	BSW 2	BSW 3	BSW 4	BSW 7	BSW 8	BSW 9
PSW 1	1																
PSW 2	0.81	1															
PSW 4	0.80	0.80	1														
PSW 5	0.96	0.81	0.84	1													
PSW 7	0.89	0.79	0.77	0.91	1												
PSW 9	0.92	0.84	0.80	0.91	0.88	1											
PSW 10	0.96	0.82	0.81	0.97	0.93	0.91	1										
PSW 11	0.91	0.80	0.80	0.94	0.95	0.88	0.94	1									
PSW 12	0.96	0.80	0.82	0.97	0.90	0.92	0.97	0.92	1								
PSW 13	0.82	0.68	0.83	0.84	0.79	0.79	0.81	0.78	0.83	1							
BSW 1	0.75	0.67	0.57	0.72	0.73	0.72	0.74	0.74	0.74	0.56	1						
BSW 2	0.75	0.67	0.64	0.74	0.77	0.74	0.76	0.76	0.75	0.63	0.90	1					
BSW 3	0.68	0.60	0.60	0.70	0.70	0.65	0.69	0.71	0.68	0.60	0.83	0.87	1				
BSW 4	0.75	0.70	0.66	0.77	0.81	0.77	0.77	0.79	0.78	0.66	0.88	0.93	0.85	1			
BSW 7	0.73	0.68	0.64	0.74	0.72	0.73	0.74	0.74	0.73	0.59	0.85	0.91	0.85	0.89	1		
BSW 8	0.76	0.66	0.62	0.76	0.77	0.74	0.78	0.78	0.78	0.61	0.92	0.94	0.87	0.92	0.91	1	
BSW 9	0.77	0.69	0.63	0.76	0.78	0.74	0.78	0.78	0.76	0.62	0.90	0.94	0.85	0.94	0.89	0.94	1

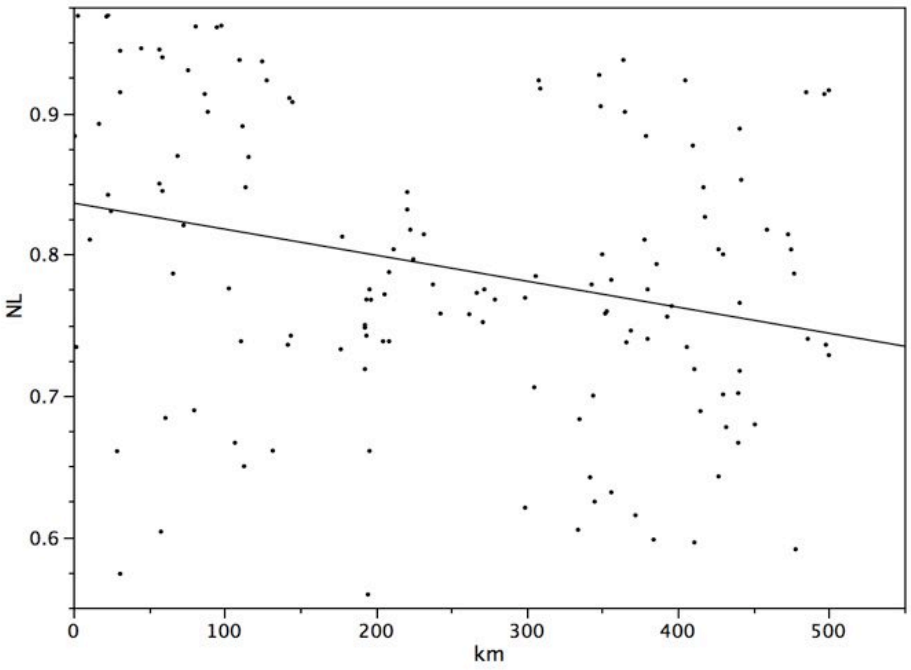


Figure 3.3. Linear regression of pair-wise genetic similarity coefficient (Nei and Li's – NL) by pairwise distance between 7 BSW and 10 PSW populations in New York. Regression equation: NL = 0.836 – 0.000018*km; $r^2 = 0.08$, $P < 0.0001$.

Table 3.10. Summary of geographic distances (km) between the ten PSW populations and seven BSW populations in New York whose coefficients of genetic similarity (NL) are shown in **Table 3.8**.

	PSW 1	PSW 2	PSW 4	PSW 5	PSW 7	PSW 9	PSW 10	PSW 11	PSW 12	PSW 13	BSW 1	BSW 2	BSW 3	BSW 4	BSW 7	BSW 8	BSW 9
PSW 1	0																
PSW 2	378	0															
PSW 4	212	430	0														
PSW 5	95	473	221	0													
PSW 7	17	386	197	87	0												
PSW 9	405	59	427	497	410	0											
PSW 10	81	459	232	22	76	485	0										
PSW 11	31	350	225	125	45	379	110	0									
PSW 12	98	475	223	3	89	500	23	128	0								
PSW 13	73	451	221	23	66	477	11	103	25	0							
BSW 1	193	440	31	193	177	441	205	209	194	195	0						
BSW 2	271	107	342	366	279	142	352	243	369	345	349	0					
BSW 3	335	58	411	430	344	113	415	305	432	384	418	69	0				
BSW 4	193	440	29	194	178	441	206	209	196	196	1	348	417	0			
BSW 7	406	61	427	498	411	2	486	380	500	478	442	143	114	441	0		
BSW 8	262	132	299	353	267	144	343	238	356	334	309	57	116	308	145	0	
BSW 9	299	80	356	393	306	111	380	272	396	372	365	31	57	364	112	59	0

Although analysis of genetic similarity alone showed high overall similarities among the sampled populations (**Figure 3.2**), phylogenetic analysis of the RAPD data strongly indicates that each SW species is monophyletic (**Figure 3.4**). The separation of the two species was recovered in 100% of the bootstrap analyses for both tree construction methods (MP & NJ). Relationships among individuals within species groups were generally weakly supported with the following exceptions. Both analyses suggest that PSW populations 4 and 13 are monophyletic with bootstrap values of 82 (MP) and 75 (NJ) (221 km apart). Parsimony analysis (MP) revealed that PSW populations 7 and 11 (45 km apart) are basal grade with 11 sister to the remaining pale individuals. However, NJ analysis resolved these two populations as taxa with this clade sister to the remaining pale individuals (bootstrap value = 74). Although pair-wise analysis of genetic similarity suggests a lack of diversity among the SW populations, phylogenetic analysis did not offer any indication of hybridization between the two species.

Plant Morphologies and Genetic Diversity. Mean NL genetic similarity values for each site were not found to be strongly correlated with any component of SW morphological variation. PCA using original morphology variables and including mean NL values for each site revealed that genetic diversity explained a substantial portion of variation only in the third principal component, which accounted for merely 18% of the morphological variation between sampled sites.

DISCUSSION

Swallow-wort plant morphology. Plants of both SW species growing in shaded sites had larger leaves and longer internodes than plants in sunnier habitats (**Table 3.1**). This finding is consistent with morphological observations of PSW made by Sheeley

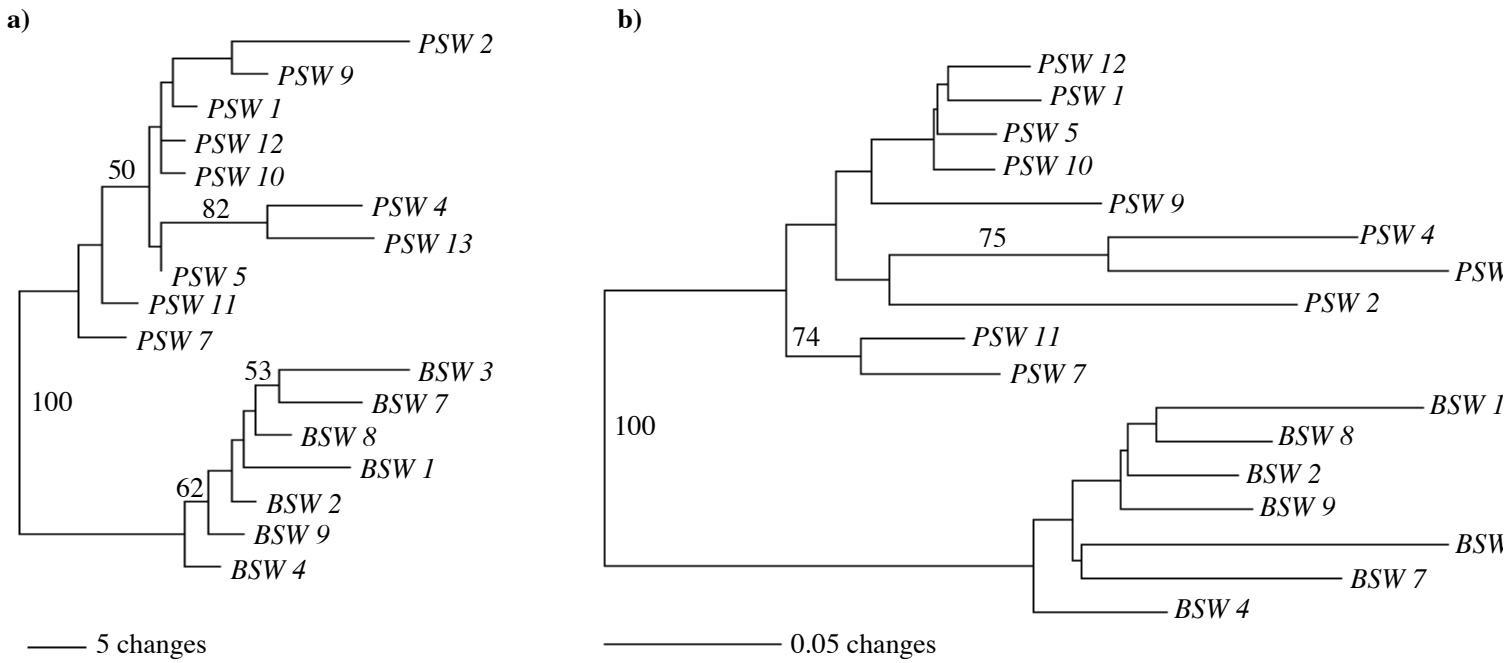


Figure 3.4. Comparison of phylogenetic trees of PSW and BSW populations in New York created using maximum parsimony (MP, Tree 'a') and the neighbor-joining (NJ, Tree 'b') methods. For each method, a bootstrap analysis with 1,000 replicates was used; bootstrap values are shown above only when the value > 50 for the given node

(1992) at a forest gap site in central NY, and reflects typical plant physiological responses to growing in shaded conditions (Claridge and Franklin 2002; Dudley and Schmitt 1996; Fitter and Hay 1987; Schweitzer and Larson 1999; Weinig 2000). However, there was variability in internode length responses to light availability (**Table 3.1**). Several PSW plants in full sun locations had elongated internodes comparable to those of plants in shaded habitats at other sites. A comparison of overall plant morphologies of both SW species from full sun sites found that BSW plants had 14% ($P < 0.05$) shorter internodes than PSW plants in similar conditions. Schweitzer and Larson (1999) found that *Lonicera japonica* Thunb. vines growing in open fields sometimes assumed a trailing growth habit with longer internodes, which was hypothesized to allow for rapid colonization of climbing supports. Furthermore, Claridge and Franklin (2002) found that otherwise typical patterns of plasticity of *Microstegium vimineum* in response to light and moisture were altered when the plant was in competition with other species in an open field.

Morphologically, PSW leaves were longer than those of BSW, and a minimal increase in leaf widths in the former species suggests a generally narrower leaf shape (**Table 3.2**). Lauvanger and Borgen (1998) also reported that PSW leaves were larger than BSW, though their work with populations in Norway indicates that typical leaves of the species there are up to 1 cm narrower and almost 2 cm shorter.

Swallow-wort species and soils. Overall, the soil pH of sites sampled (PSW: 5.2 – 7.6; BSW: 5.2 – 7.2) was lower than the alkaline soils typically reported as being favored by the SW species (DiTommaso et al. 2005b; Weston et al. 2005). However, our results were consistent with previous findings at infested sites in central NY (Sheeley 1992; Smith 2006). Sites with PSW infestations had much higher levels of calcium and aluminum, and slightly higher pH levels (**Table 3.2**; pH = 6.8 versus pH

= 6.0 for BSW sites). The high calcium levels found in soils associated with PSW supports previous work suggesting that PSW prefers calcareous soils (DiTommaso et al. 2005b; Sheeley 1992; Smith 2006). Analysis of variations between sites for soil characteristics revealed that the largest portion of variation between sites was due to pH and magnesium levels (**Table 3.5**). Mg availability is strongly related to pH, with magnesium levels decreasing as pH levels fall below 4-5 (Brady and Weil 2002). Mg levels found at our sampled sites are consistent with previous reports that sites infested by PSW had substantially higher levels of this nutrient than was typical for the surrounding area (Smith 2006).

Overall, SW plant height and the number of nodes per plant were negatively correlated with pH levels (**Table 3.4**; **Figure 3.5**). Sites characterized by taller plants with a greater number of nodes were found to have low pH and Mg levels (**Appendix II, Figure B2**). However, Smith (2006) reported that variability in soil pH levels had no significant effect on several PSW growth parameters at a site sampled in central New York. However, the range of pH levels reported by Smith (2006) was limited (6.7 ± 0.05), and may not represent the ability of the species to thrive at sites with much lower pH levels (e.g. 5.2).

Swallow-wort species and climate. In spite of differences in climate (particularly temperature and precipitation) of sampled SW sites (**Table 3.5**), climate was not found to be a strong determinant of swallow-wort plant morphology (**Table 3.6**). Plant height and internode length were both found to be positively correlated with precipitation levels, while leaf size was positively correlated with the duration of the growing season (**Appendix II, Figures B3a – B3c**).

Genetic diversity of swallow-worts. Phylogenetic analysis determined that PSW and

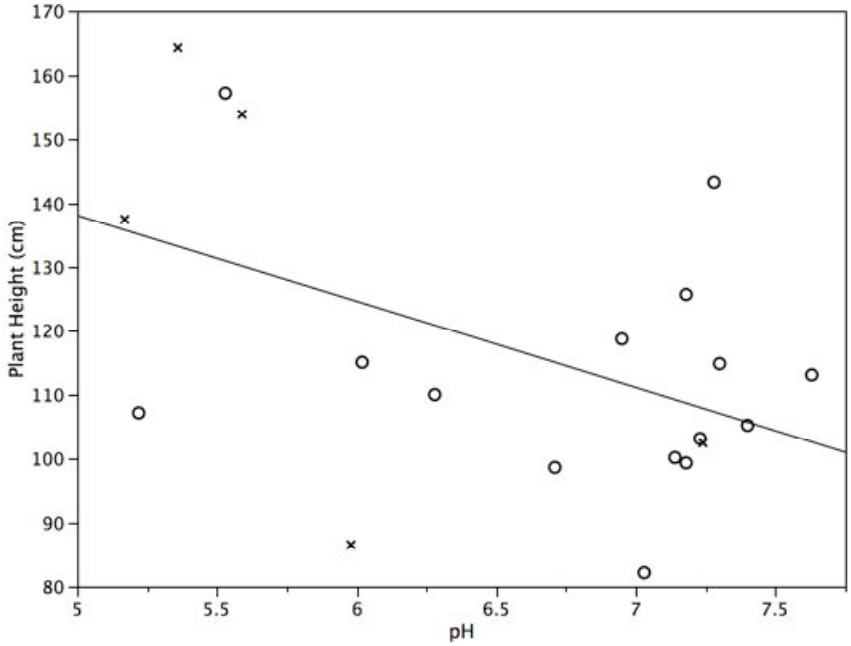


Figure 3.5. Linear regression of SW plant height on soil pH level. BSW sites are marked with an 'X' while PSW sites are marked with an 'O' (for more detailed analysis of site responses see Appendix II, Figure 2). Regression expression: plant height = 205.73 – 13.52*pH; $r^2 = 0.24$, P = 0.0297.

BSW are monophyletic species, the first molecular evidence for this taxonomic division. However we also found that there was substantial intra-species genetic similarity. Neither maximum parsimony nor neighbor joining methods could conclusively reveal phylogenetic divisions within the species themselves (**Figure 3.6**). Similarly, subsequent analysis of genetic similarity using NL coefficients found that within species similarity was greater than between species similarity (**Table 3.9**).

Genetic similarity of swallow-worts. Research has shown that introduced populations of plant species are often less genetically diverse than those in native ranges (Barrett and Richardson 1986; Durka et al. 2005; Meekins et al. 2001; Tsutui et al. 2000;

Wilson and Rapson 1995; Xu et al. 2003). Reduced genetic diversity in introduced SW population could be due to their reproductive systems, i.e. vegetative reproduction and/or autogamy (Leimu 2004; Lumer and Yost 1995). Richardson and Pysek (2006) proposed that autogamy might be advantageous as it could provide reproductive assurances for smaller populations of introduced species if the plants had a "general purpose genotype."

However, the lack of genetic diversity of SW populations in New York could also be due to their introduction history. If the number of introductions was minimal, or if introduced plants were drawn from the same source populations, then it is likely that reduced genetic variation would result (Barrett and Kohn 1991; Sakai et al. 2001; Tsutsui et al. 2000).

Dispersal and its implications. Cappuccino et al. (2002) found that the maximum distance PSW was naturally dispersed (given a windspeed of 11.2 km/hr) was 18 m. Also, the establishment of new SW patches has been estimated to take several years due to slow initial growth and a lag phase before reproduction may take place (Cappuccino 2004). Yet, in the last few decades, spread of these two SW species has occurred more rapidly than empirical studies might suggest (DiTommaso et al. 2005a). Biological invasions may occur much faster than predicted by rates of natural migration, in part due to an underestimation of rates of long-distance dispersal (Cain et al. 2000; Richardson and Pysek 2006). Rapid range expansion, long lag times (during which adaptive evolution might take place) and multiple introductions could increase the genetic diversity of resulting populations (Barrett and Husband 1990; Barrett and Husband 1991; Durka et al. 2005; Ellstrand and Schierenbeck 2000; Lavergne and Molofsky 2007; Levin 2003).

It is also possible that the rapid spread of SWs in recent decades could be due to human intervention, especially given the likelihood that the species were intentionally introduced (Hodkinson and Thompson 1997; Monanchino 1957; Sheeley and Raynal 1996). Considering the self-compatibility of SW species, and that the ranges of introduced *Vincetoxicum* have begun to overlap causing the two species to co-occur in some habitats (A DiTommaso, Personal communication), it is possible that interspecific hybridization could occur – though our molecular evidence does not indicate that this has occurred yet (Ellstrand and Shierenbeck 2000; Hollingsworth et al. 1999).

Ecological Context. Although our research could not explicitly address whether morphological variations apparent in swallow-wort populations were specifically adaptive, our analysis of patterns of variations between the sites suggests that these two invasive species are not successful merely because they are opportunistic. Initial establishment of SW is thought to be facilitated by disturbances or a lack of highly competitive neighbors (Lawlor 2000; Sheeley 1992). However, SWs have proven adept at expanding into habitats both rich in and lacking resources, as well as sites with low and high diversities of existing vegetation (Ladd and Cappuccino 2005; Lawlor 2000; Smith 2006). This suggests that SWs may be rare invasive species that have greater plasticity in adaptive competitive traits, and that this then allows the two species to generally out-compete non-invasive and native species in a range of environments (Burns and Winn 2006; Richards et al. 2006).

The priority for SW research in the future should be two-fold. Firstly, comparative studies with populations in the native ranges of both species are necessary to better elucidate the evolution of genetic and phenotypic changes in introduced populations (Hierro et al. 2005; Meekins et al. 2001). Secondly,

comparison with related exotic species that have not become invasive in North America would allow the determination of specific traits that are promoting the invasiveness of SWs (Burns and Winn 2006).

Summary. Our work has provided the first molecular evidence that the two SW species are monophyletic, and thus are genetically distinct from one another. However, intra-species genetic diversity levels were lower than expected, and we found a weakly negative correlation between geographic distance separating populations and their genetic similarity.

Full sun, open field sites appear to be favored by BSW plants, as the majority of sites found to be heavily invaded by the species were in full sun. BSW plants in our study were 5% taller, had 33% more nodes, and had 32% shorter internodes than sampled PSW plants. Sites characterized by high Ca and Al levels and slightly basic pH levels were favored by PSW plants. Overall, averaged across SW species, leaf growth of SW plants sampled was especially favored by high soil nitrate levels, while plant elongation was stimulated at sites with slightly acidic soil pH levels.

Principal components analysis suggested that genetic diversity did not explain any significant portion of morphological patterns found for sampled SW populations in New York. Rather, soil pH and precipitation levels explained much of the morphological variability of SWs.

APPENDIX II

Table B1. Location data for swallow-wort sites sampled in New York in summer 2006.

Site ID	Location (Lat/Long)	County
PSW 1	42 39.683 N 76 41.936 W	Seneca
PSW 2	40 46.397 N 72 54.258 W	Suffolk
PSW 3	42 59.810 N 76 05.592 W	Onondaga
PSW 4	44 14.457 N 75 13.455 W	St. Lawrence
PSW 5	43 13.293 N 77 35.078 W	Monroe
PSW 6	43 00.095 N 76 23.775 W	Onondaga
PSW 7	42 48.600 N 76 40.645 W	Cayuga
PSW 8	44 26.819 N 75 13.816 W	St. Lawrence
PSW 9	41 03.212 N 72 18.475 W	Suffolk
PSW 10	43 01.287 N 77 33.689 W	Monroe
PSW 11	42 25.971 N 76 29.047 W	Tompkins
PSW 12	43 13.677 N 77 37.047 W	Monroe
PSW 13	43 02.833 N 77 25.662 W	Ontario
PSW 14	43 52.706 N 76 16.427 W	Jefferson

Site ID	Location (Lat/Long)	County
BSW 1	44 12.041 N 75 36.264 W	St. Lawrence
BSW 2	41 17.989 N 73 58.204 W	Orange
BSW 3	40 44.777 N 73 35.219 W	Nassau
BSW 4	44 12.093 N 75 35.228 W	St. Lawrence
BSW 6	44 22.641 N 73 21.858 W	Essex
BSW 7	41 03.580 N 72 17.490 W	Suffolk
BSW 8	41 46.764 N 73 44.404 W	Dutchess
BSW 9	41 15.751 N 73 36.191 W	Westchester

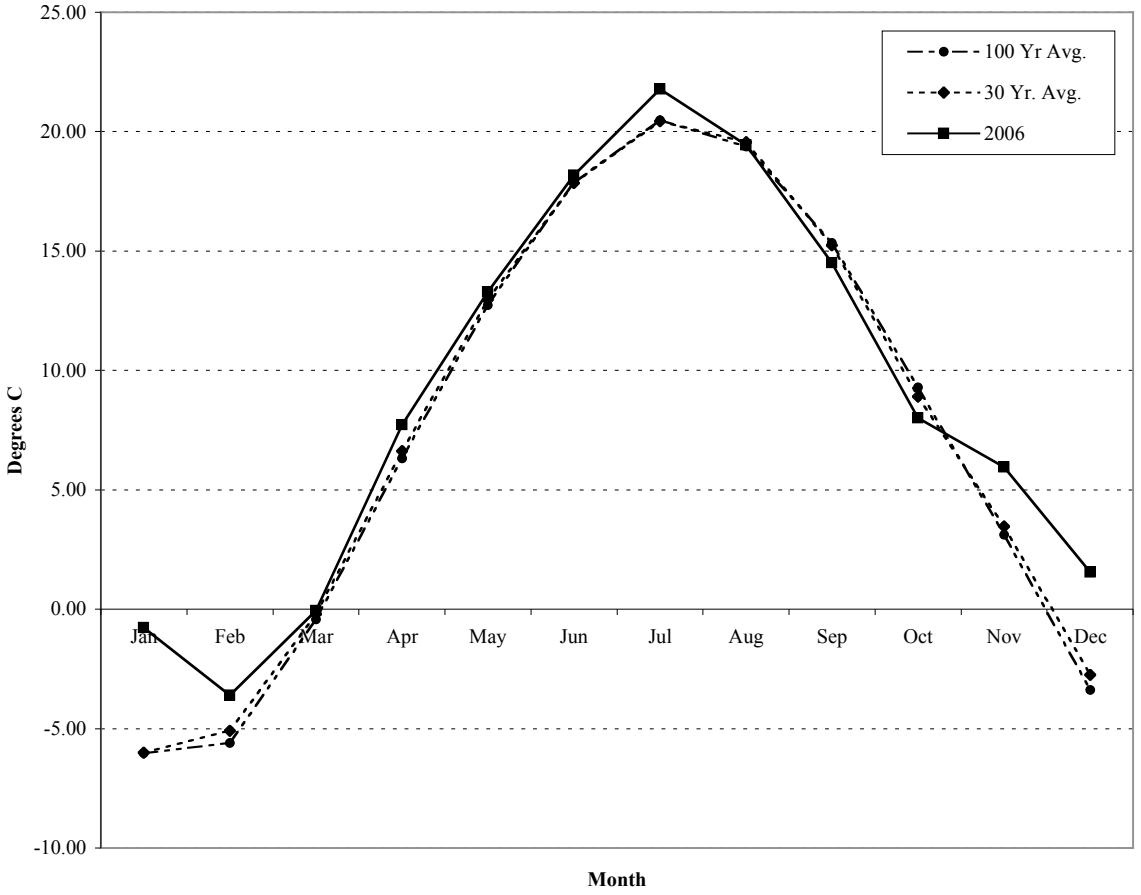


Figure B1a. Comparison of monthly temperature trends for two historical (100 yr and 30 yr) averages and data from 2006.

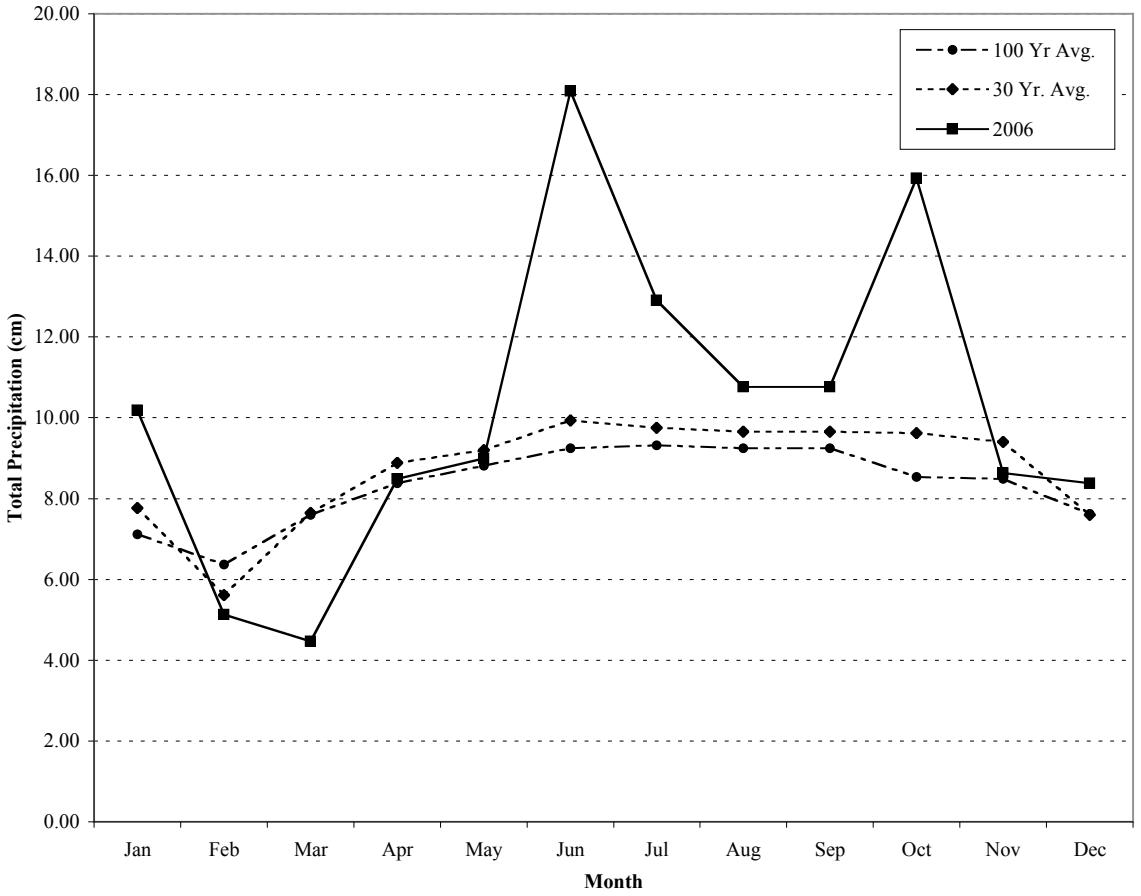


Figure B1b. Comparison of monthly precipitation trends for two historical (100 yr and 30 yr) averages and data from 2006.

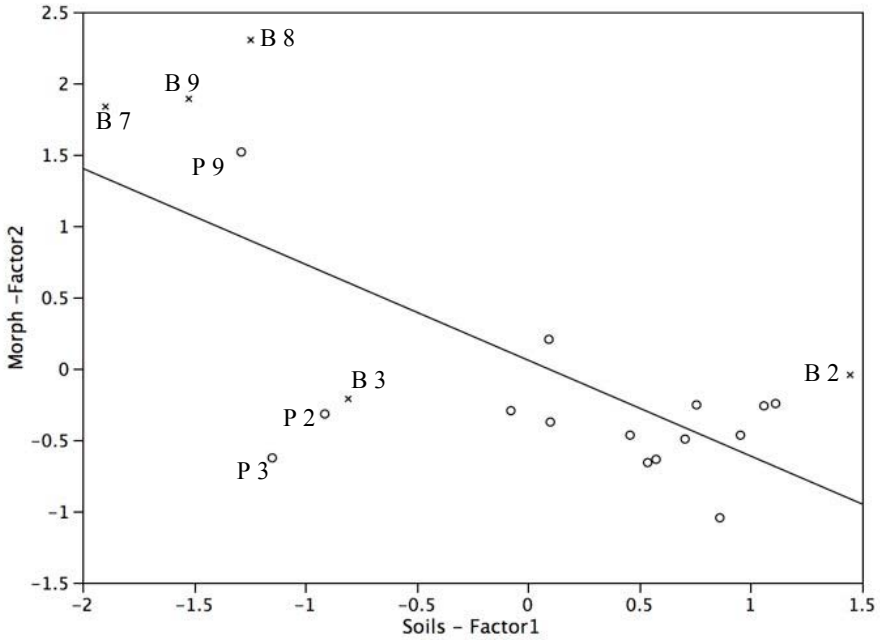


Figure B2. The second principal component of morphology (dominated by plant height & node number) as a function of the first component of soils (pH and Mg levels) (see **Table 3.7** for all loadings). $\beta_1 = -0.67$, $r^2 = 0.5$, P = 0.0005. BSW sites are indicated by an 'X,' PSW by an 'O.' Outlying or otherwise informative individual sites are indicated with a 'B_' for BSW sites and a 'P_' for PSW sites.

3a.

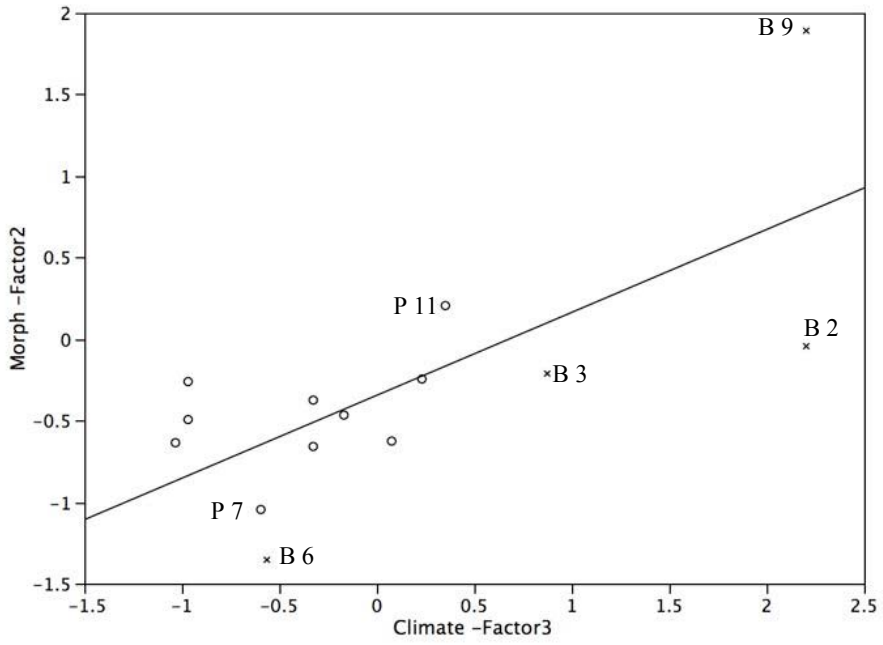
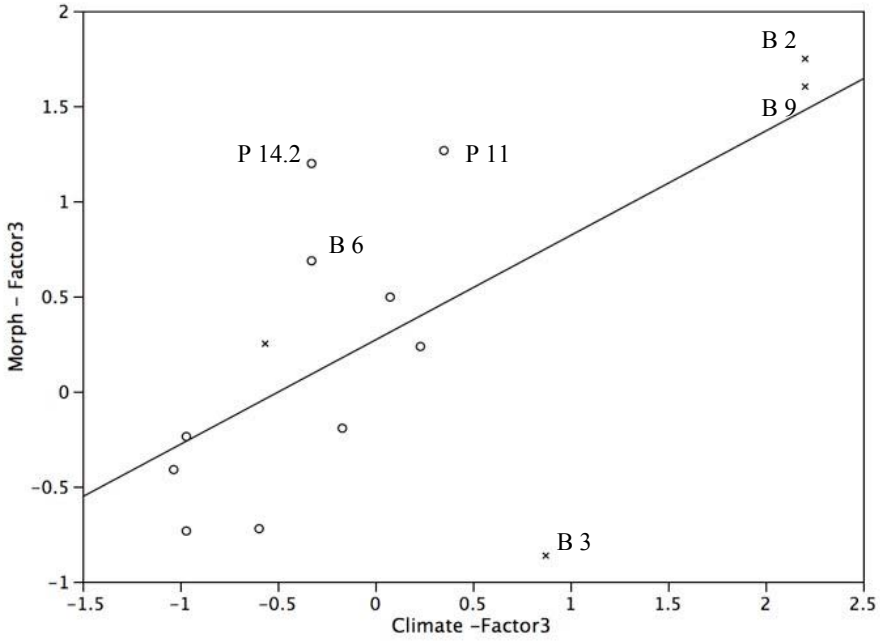
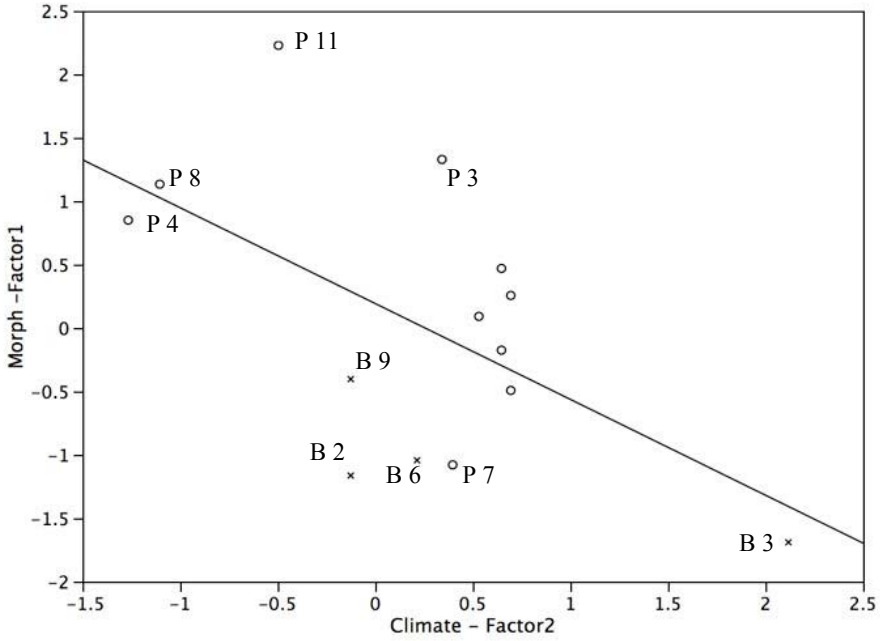


Figure B3. Relationship between morphological measurements and climate variables. **A.** The second principal component of morphology (dominated by plant height and node number) as a function of the third component of climate (historical precipitation levels and frost in date) (see **Table 3.7** for all loadings). $\beta_1 = 0.51$, $r^2 = 0.52$, P = 0.0037. **B.** The third principal component of morphology (dominated by leaf shape) as a function of the third component of climate (historical precipitation levels and frost in date). $\beta_1 = 0.55$, $r^2 = 0.43$, P = 0.0114. **C.** The first principal component of morphology (dominated by leaf width and length) as a function of the second component of climate (temperature day sampled and freeze free period). $\beta_1 = -0.76$, $r^2 = 0.33$, P = 0.0308. BSW sites are indicated by an 'X,' PSW by an 'O.' Outlying or otherwise informative individual sites are indicated with a 'B_' for BSW sites and a 'P_' for PSW sites.

3b.


B 2
B 9
P 14.2
P 11
B 6
B 3
Morph – Factor3
Climate –Factor3

3c.


P 11
P 8
P 3
P 4
B 9
B 2
B 6
P 7
B 3
Morph –Factor1
Climate – Factor2

REFERENCES

Aparicio A, S Silvestre (1985) Numeros cromosomicos para la flora Espanola 422-434. Lagascalia 13: 318-323

Bailey, J (2003) CTAB technique for DNA isolation/extraction from plant leaf samples. http://www.le.ac.uk/bl/phh4/dnaiso.htm. Last accessed 10:33 PM 5/2/2008

Baker, HG (1974) The evolution of weeds. Annual Review of Ecology and Systematics 5: 1 –24

Barrett, SCH, BC Husband (1990) The genetics of plant migration and colonization. AHD Brown, MT Clegg, Al Kahler, BS Weir, Eds. Plant Population Genetics, Breeding and Genetic Resources. Sunderland, MA: Sinaeur Associates. 449 pp

Barrett, SCH, JR Kohn (1991) Genetic and evolutionary consequences of small population size in plants: implications for conservation. DA Falk, KE Holsinger, Eds. Genetics and Conservation of Rare Plants. New York: Oxford University Press. Pp. 3-30

Barrett, SCH, BJ Richardson (1986) Genetic attributes of invading species. RH Groves, JJ Burdon, Eds. Ecology of Biological Invasions. Cambridge, UK: Cambridge University Press. Pp 21-33

Bossdorf, O, H Auge, L Lafuma, WE Rogers, E Siemann, D Prati (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144: 1-11

Bradshaw, AD (1984) The importance of evolutionary ideas in ecology – and vice versa. B Shorrocks, Ed. Evolutionary Ecology. Oxford, UK: Blackwell. Pp. 1-25

Brady, NC, RR Weil (2002) The Nature and Property of Soils. Upper Saddle River,

NJ: Prentice Hall. 960 pp

Burns, JH, AA Winn (2006) A comparison of plastic responses to competition by invasive and non-invasive congeners in the Commelinaceae. Biological Invasions 8: 797-807

Cain, ML, BG Milligan, AE Strand (2000) Long-distance seed dispersal in plant populations. American Journal of Botany 87: 1217-1227

Cappuccino N (2004) Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos 106: 3-8

Cappuccino N, Mackay R, Eisner C (2002) Spread of the invasive alien vine *Vincetoxicum rossicum*: Tradeoffs between seed dispersability and seed quality. American Midland Naturalist 148: 263-270

Chun, YJ, ML Collyer, KA Moloney, JD Nason (2007) Phenotypic plasticity of native vs invasive purple loosestrife: a two-state multivariate approach. Ecology 88: 1499-1512

Claridge, K, SB Franklin (2002) Compensation and plasticity in an invasive plant species. Biological Invasions 4: 339-347

Daehler, CC (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. Annual Review of Ecology and Systematics 34: 183-211

Davis, MA, JP Grime, K Thompson (2000) Fluctuating resources in plant communities: a general theory of invasability. Journal of Ecology 88: 528-534

Diosdado JC, F Ojeda, J Pastor (1993) CA Stace, Ed. IOPB Chromosome Data 5. Newsletter of the International Organization of Plant Biosystematics 20: 6-7

DiTommaso A, Brainard DC, Webster BR (2005a) Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration. Canadian Journal of Botany 83: 102-110

DiTommaso A, Lawlor FM, Darbyshire SJ (2005b) The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [=*Vincetoxicum nigrum* (L.) Moench]. Canadian Journal of Plant Sciences 85: 243-263

Doyle, JJ (1991) DNA protocols for plants. G Hewitt, AWB Johnson, JPW Young, Eds. Molecular Techniques in Taxonomy. NATO Cell Biology ASI Series H, Cell Biology Vol 57. Pp 283-293

Dudley, SA, J Schmitt (1996) Testing the adaptive plasticity hypothesis: density dependent selection on manipulated stem length in *Impatiens capensis*. American Naturalist 147: 445-465

Durka, W, O Bossdorf, D Prati, H Auge (2005) Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. Molecular Ecology 14: 1697-1706

Ellison, AM, HL Buckley, TE Miller, NJ Gotelli (2004) Morphological variation in *Sarracenia purpurea* (Sarraceniaceae): geographic, environmental, and taxonomic correlates. American Journal of Botany 91: 1930-1935

Ellstrand, NC, KA Shierenbeck (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97: 7043-7050

Fitter, AH, RKM Hay (1987) Environmental Physiology of Plants, 2nd Ed. San Diego, CA: Academic Press. 423 pp

Gammon, MA, JL Grimsby, D Tsirelon, R Kesseli (2007) Molecular and morphological evidence reveals introgression in swarms of the invasive taxa *Fallopia japonica*, *F. sachalinensis*, and *F.* x *bohemica* (Polygonaceae) in the United States. American Journal of Botany 94: 948-956

Gotelli, NJ, AM Ellison (2004) A Primer of Ecological Statistics. Sunderland, MA: Sinaeur Associates. 492 pp

Hierro, JL, JL Maron, RM Callaway (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native ranges. Journal of Ecology 93: 5-15

Hodkinson, DJ, K Thompson (1997) Plant dispersal: the role of man. The Journal of Applied Ecology 34: 1484-1496

Hollingsworth, ML, JP Bailey, PM Hollingsworth, C Ferris (1999) Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). Botanical Journal of the Linnean Society 129: 139-154

Hotchkiss, EE, A DiTommaso, DC Brainard, CL Mohler (2008) Survival and performance of the inasve vine *Vincetoxicum rossicum* (Apocynaceae) from seeds of different embryo number under two light environments. American Journal of Botany 95: 447-453

Joliffe, IT (2002) Principal Components Analysis, 2nd Ed. New York, NY: Springer. 502 pp

Ladd, D, N Cappuccino (2005) A field study of seed dispersal and seedling performance in the invasive exotic vine *Vincetoxicum rossicum*. Canadian Journal of Botany 83: 1181-1188

Lamboy, WF (1994) Computing genetic similarity coefficients from RAPD data: the effects of PCR artifacts. PCR Methods and Applications 4: 31-37

Lauvanger EG, Borgen L (1998) The identity of Vincetoxicum in Norway. Nordic Journal of Botany 18: 353-364

Lavergne, S, J Molofsky (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proceedings of the National Academy

of Sciences 104: 3883-3888

Lawlor FM (2000) Herbicidal treatment of the invasive plant *Cynanchum rossicum* and experimental post control restoration of infested sites. MSc Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY. 126 pp

Lawlor FM, Raynal DJ (2002) Response of swallow-wort to herbicides. Weed Sci 50: 179-185

Lee, CE (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17: 386-391

Lehman, A, N O'Rourke, E Stepanski, L Hatcher (2005) JMP for Basic Univariate and Multivariate Statistics: A Step-by-step guide. Cary, NC: SAS Institute, Inc. 481 pp

Leimu, R (2004) Variation in the mating system of *Vincetoxicum hirundinaria* (Asclepiadaceae) in peripheral island populations. Annals of Botany 93: 107-113

Levin, DA (2003) Ecological speciation: lessons from invasive species. Systematic Botany 28: 643-650

Lumer C, Yost SE (1995) The reproductive biology of *Vincetoxicum nigrum* (L.) Moench (Asclepiadaceae), a Mediterranean weed in New York state. Bulletin of the Torrey Botanical Club 122: 15-23

Maron, JL, M Vila, R Bommarco, S Elmendorf, P Beardsley (2004) Rapid evolution of an invasive plant. Ecological Monographs 74: 261-280

McArthur, ED, R Van Buren, SC Sanderson, KT Harper (1998) Taxonomy of *Sphaeromoeria*, *Artemisia* and *Tanacetum* (Compositae, Anthemidae) based on randomly amplified polymorphic DNA (RAPD). The Great Basin Naturalist 58: 1-11

Meekins, JF, HE Ballard, BC McCarthy (2001) Genetic variation and molecular biogeography of a North American invasive plant species (*Alliaria petiolata*, Brassicaceae). International Journal of Plant Sciences 162: 161-169

Monachino J (1957) Cynanchum in the New York area. Bulletin of the Torrey Botanical Club 84: 47-48

Moore RJ (1959) The dog-strangling vine *Cynanchum medium*, its chromosome number and its occurrence in Canada. Canadian Field Naturalist 73: 144-147

Nei, L, WH Li (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76: 5269-5273

Nei, M, T Maruyama, R Chakraborty (1975) The bottleneck effect and genetic variability in populations. Evolution 29(1): 1-10

New York State Climate Office (2008) Climate of New York. http://nysc.eas.cornell.edu/climate_of_ny.html. Last accessed 9:57 PM 17/2/2008

NOAA Global Survey of the Day – GSOD (2008) http://www7.ncdc.noaa.gov/CDO/cdoselect.cmd. Last accessed 5:23 PM 18/2/2008

NOAA Logs (2008) Climatological Observation Forms. http://www7.ncdc.noaa.gov/IPS/getcoopstates.html. Last accessed 5:26 PM 18/2/2008

NOAA NCDC (2000) Climatography of the United States No 20. http://www.ncdc.noaa. gov/oa/ mpp/freedata.html. Last accessed 5:30 PM 18/2/2008

NOAA NCDC (2008) Freeze/Frost Data – CLIM20 Supp No 1. http://www.ncdc.noaa.gov/oa/ mpp/freedata.html. Last accessed 5:32 PM 18/2/2008

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? Trends in Ecolology and Evolution 20(9). DOI 10.1016/j.tree.2005.06.001

Richards CL, Bossdorf O, Muth N, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9: 981-993

Richardson, DM, P Pysek (2006) Plant invasions: merging the concepts of species invasiveness and community invasability. Progress in Physical Geography 30: 409-431

Sakai, AK, FW Allendorf, JS Holt, DM Lodge, J Molofsky, KA With, S Baughman, RJ Cabin, JE Cohen, NC Ellstrand, DE McCauley, P O'Neil, IM Parker, JN Thompson, SW Weller (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305-332

Schweitzer, J, KC Larson (1999) Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. Journal of the Torrey Botanical Society 126: 15-23

Sheeley SE (1992) Life history and distribution of *Vincetoxicum rossicum* (Asclepiadaceae): An exotic plant in North America. MSc Thesis, State University of New York College of Environmental Science and Forestry, Syracuse, NY. 126 pp

Sheeley SE, Raynal DJ (1996) The distribution and status of species of *Vincetoxicum* in eastern North America. Bulletin of the Torrey Botanical Club 123: 148-156

Smith LL (2006) Arbuscular mycorrhizal fungi and the competitiveness of the invasive vine pale swallow-wort (*Vincetoxicum rossicum*). MSc Thesis, Cornell University, Ithaca, NY. 85 pp

Tsutui, ND, AV Suarez, DA Holway, TJ Case (2000) Reduced genetic variation and

the success of an invasive species. Proceedings of the National Academy of Sciences 97: 5948-5953

Weinig, C (2000) Differing selection in alternative competitive environments: shade avoidance responses and germination timing. Evolution 54: 124-136

Weston LA, Barney JN, DiTommaso A (2005) A review of the biology, ecology and potential management of three important invasive perennials in New York state: japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant Soil 277: 53-69

Wilson, JB, GL Rapson (1995) The genetics of naturalization: a comparison of morphological variation within and between populations of *Agrostis capillaries* L. as an exotic in New Zealand and as a native in Britain. New Zealand Journal of Ecology 19: 195-202

World Meteorological Organization (2008) World Weather Information Service. http://www.worldweather.org. Last accessed 9:56 PM 17/2/2008

Xu, CY, WJ Zhang, CZ Fu, BR Lu (2003) Genetic diversity of alligator weed in China by RAPD analysis. Biodiversity and Conservation 12: 637-645